Guest Editorial

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Quorum Sensing from Two Engineers' Perspectives

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Abstract: Quorum sensing, a bacterial process for coordinating community behavior, has inspired scientists to engineer cell-cell communication for diverse applications. Fundamental knowledge of the molecular underpinnings of quorum sensing systems enabled engineers to rewire quorum sensing circuits in order to alter quorum sensing processes, program control of bacterial populations, and engineer cell-cell communication. Further, scientific advancements from diverse engineering disciplines have contributed to the design of devices enabling new modes of manipulating or communicating with biological cells. This perspective reviews early and current developments in engineering cell-cell communication and its applications. Influence of the quorum sensing field on the authors, both engineers, is briefly discussed.

Keywords: cell-cell communication · electrochemistry · microbial consortia · synthetic biology · quorum sensing

1. Introduction

"Quorum sensing", the term bestowed upon the molecular "communication" process that allows unicellular bacteria to coordinate phenotype with their neighbors through the secretion and recognition of signaling molecules called autoinducers, has inspired many scientists and engineers to seek fundamental understanding, as well as innovative pathways for application. Not long after the term was first coined by Fuqua, Winans, and Greenberg,[1] engineers, inspired by these natural processes, began to manipulate signaling pathways for altering communication and even programmed control of bacterial populations. As the fundamental knowledge underpinning quorum sensing (QS) has grown, so has the sophistication of these engineered systems and the breadth of possible applications (Figure 1). An exciting area of recent and current work aims to engineer QS pathways for controlling cocultures or microbial consortia and for connecting biology with electronics. This perspective reviews the early architectures that enabled these developments as well as their influence on a few selected engineered QS systems of particular interest as well as the current state of the field in these areas.

2. Comparison of AHL and AI-2 QS Systems

Two of the most well-established classes of QS systems include the autoinducer-1 and −2 (AI-1 and AI-2) systems of Gram-negative bacteria.[7] In the prototypical QS process, cells produce molecular signals (autoinducers) as a natural consequence of their growth, leading to an increase in the extracellular concentration commensurate with increased cell density (hence, the connection to a voting "quorum"). A specific concentration of autoinducer (corresponding to a specific cell density) activates gene expression and synchronizes phenotype across the population. In the canonical AI-1 (also referred to as AHL owing to the acyl-homoserine lactone autoinducer) system from *Vibrio fischeri*, the signal synthase LuxI synthesizes the autoinducer. Once the threshold concentration of AHL is reached, the receptor LuxR binds the signal and activates gene expression from the *lux* operon. This includes additional expression of the synthase LuxI, creating a feed-forward control loop. Homologous, species-specific systems exist in several other species. Engineers seized the opportunity to engineer cell-cell communication using AHL

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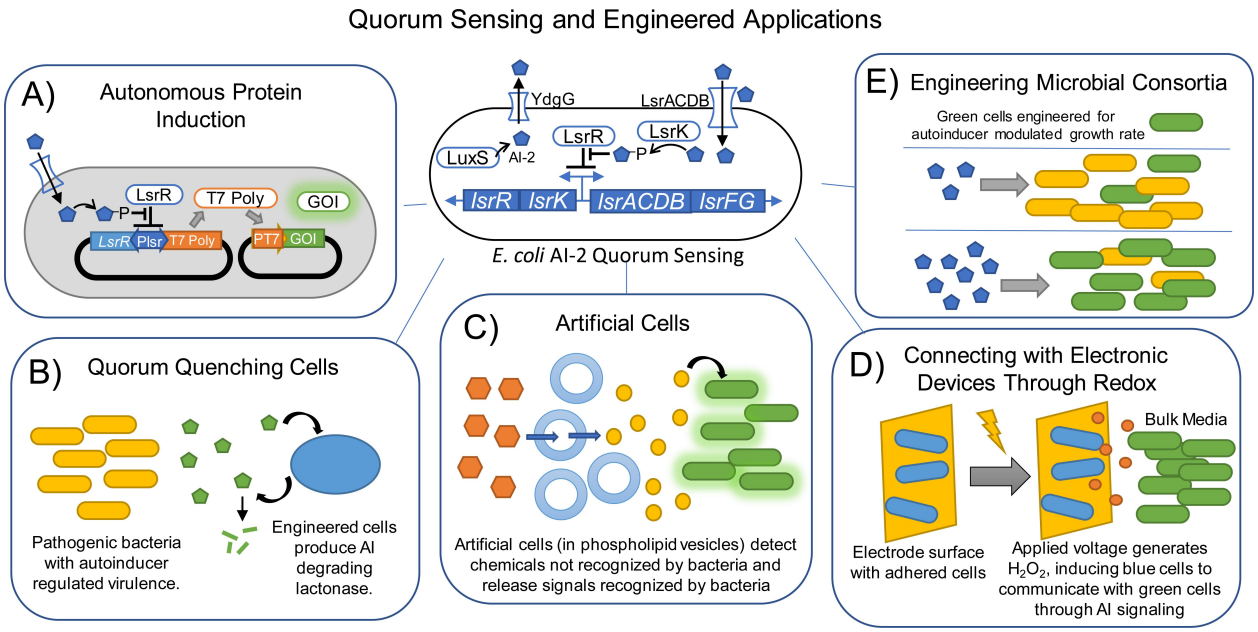

Figure 1. Applications of engineered quorum sensing systems. A native quorum sensing system, the AI-2 system from *E. coli* is depicted in the center. The AI-2 quorum sensing system or cell-cell communication more generally, were used to engineer the systems shown. **A)** A two-plasmid system containing parts of the AI-2 system was designed for autonomous induction of a gene of interest (GOI) for recombinant protein production in *E. coli*.[2] **B)** Engineered mammalian quorum quenching cells can interfere with quorum sensing in pathogenic bacteria.[3] **C)** Artificial cells were designed to detect a signal not recognized by bacteria and secrete a signal recognized by bacteria.[4] **D)** An electronic device was designed to induce electrode-adhered cells to synthesize autoinducers for communicating with bulk cells.[5] **E)** Cells engineered for autoinducer-modulated cell growth rate can be used to modulate culture composition.[6]

systems, as only the signal synthase, receptor, and cognate promoter with a specific gene of interest need to be cloned into the desired host organism, usually a laboratory strain of *E. coli*. These facultative anaerobes were ideally suited as they do not natively express the synthase or regulator genes. Thus, populations of cells could be constructed that could synthesize or respond to AHL molecules using the components described as a base resulting in a variety of population dynamics depending on innovations in circuit design.[8–11] Importantly, the AHLs could pass through the Gram-negative cell membrane owing to their alkyl chains, while the AI-2 molecules were significantly more hydrophilic[12] and required phosphorylation[13–16] and/or bromination[17] for transport or subsequent signal transduction. Thus, the relative ease of the AHL systems along with the explosion of genetic circuits[18] lead to many innovative tools using QS components including various logic gates,[19] oscillators,[20] and clocks.[21,22]

In the AI-2 QS system of *E. coli* (Figure 1), LuxS synthesizes AI-2 which is secreted by YdgG out of the cell.[23–25] Once the extracellular concentration of AI-2 has increased past a specific threshold, LsrACDB transports AI-2 into the cell. There, it is phosphorylated by LsrK. Phosphorylated AI-2 binds the repressor of the *lsr* operon, LsrR, and relieves repression. Interestingly, owing to the two CRP binding sites that are proximal within the regulatory region, LsrR repression consists of two sets of dimers which can fold over onto each other creating a tetrameric assembly (akin to DeoR-type repressors[26]), wherein dimers of the LsrR repressor can bind AI-2P contributing to a cascaded de-repression that can consist of many potentially controllable states.[27,28] Then LsrFG degrade the autoinducer returning the carbon backbone to endogenous metabolic activity.[29] Early on, it was also discovered that AI-2, unlike the AHL signaling molecules, was recognized and produced by multiple species. Indeed, AI-2 is a family of isomers with or without functionalization provided by cyclization, boronation, or phosphorylation.[13,15–17] From an engineering perspective, this made it an especially exciting target for designing user-specific applications, but the system proved to be relatively complicated for engineering compared to the AHL systems. First, it consists of more componentry than in AHL systems, including a transporter. Further, it was discovered that the molecule LuxS synthesizes is 4,5-dihydroxy-2,3-pentanedione (DPD), which is not very stable and spontaneously transforms into a collection of other molecules which are believed to be recognized by the cell as the autoinducer (AI-2).[16,17] In *Vibrio* species, the autoinducer is recognized only when it is bound to boron[17] (which exists in its native marine environment), while in *E. coli* boron is not part of the recognized autoinducer structure. Finally, it also was recognized that LuxS, as part of the activated methyl cycle, is an integral part of metabolism. That is, within the activated methyl cycle, LuxS converts S-ribosylhomocysteine to homocysteine and produces DPD, the AI-2 precursor, as a byproduct, thus playing a role in both QS

and metabolism.[30] Notably, AHLs are synthesized by the transfer of an acyl group to S-adenosylmethionine, another molecule in the activated methyl cycle.[31] Further research over the years has demonstrated that the AI-2 QS system is linked to metabolism at multiple points. For instance, the *lsr* operon is subject to carbon catabolite repression and is repressed when the cells are grown in the presence of glucose.[13] The rate of synthesis of AI-2 is dependent on cell growth rate.[32] Finally, the *lsr* system is linked to the phosphoenolpyruvate phosphotransferase system (PTS) which transports PTS substrates including glucose.[33,34] This suggests that information about metabolism is integrated into the AI-2 QS process. Indeed, its production level was seen to be reduced when metabolic activity was redirected into the synthesis of heterologous proteins.[35]

The complexities of the AI-2 QS system as compared to the AHL systems, have provided many interesting nodes for altering the QS processes (discussed further in the next section). In hindsight, some of the early work on AI-2 QS gave hints to the breadth of work that came later showing that QS processes are affected by their environment and/or the presence of other species. Knowledge of intricate signal transduction processes are particularly relevant now given the explosion of research directed towards microbiomes and microbial consortia, where it is very difficult to understand the contributions of individual species which may behave differently when grown outside their native environments and when separated from the consortia.

3. Engineering the AI-2 QS Pathway

The *E. coli* AI-2 QS pathway has been rewired both to precisely control QS responses of the engineered cells (for example, to enable autonomous induction of protein expression[2]) and to design engineered cells capable of manipulating QS processes within neighboring wild type populations (for example, through rapid uptake of and depletion of AI-2[36]). A few examples of each and their applications are discussed here.

An early demonstration of manipulating the AI-2 QS pathway for an applied goal showed that the AI-2 QS pathway could be rewired to enable autoinduction of a gene of interest.[2] A low copy plasmid encoding T7 RNA polymerase controlled under the *lsr* promoter allowed autonomous activation of a T7 inducible promoter with a gene of interest contained on a common pET expression plasmid (Figure 1). The system was analyzed both with and without *lsrR* expression on the low copy plasmid, which is natively expressed under the bidirectional *lsr* promoter. It was found that inclusion of *lsrR* resulted in a more tightly regulated switch to production of the gene of interest. Since then, several studies in *E. coli* have demonstrated that through manipulation of different components of the AI-2 QS pathway, the response to AI-2 can be fined-tuned in order to, for example, harness the natural variation in the QS response to design cultures with programmed subpopulations,[37] increase uniformity of autoinduced protein expression across the population,[38] design AI-2 reporter cells with higher sensitivity to AI-2,[39] and engineer cells where synthesis of the gene of interest in response to AI-2 is also regulated by a second external cue in order to tightly control the response.[40] The last example could be applied towards engineered probiotic bacteria, where it may be important to tightly regulate synthesis of the gene of interest. In that work, authors demonstrated controlled expression of human granulocyte macrophage colony stimulating factor in a probiotic strain.

Engineered cells can also be designed to manipulate QS processes of wild type cultures. There are numerous examples of quorum quenching, where a QS signal is removed, by sequestration or degradation, in order to alter QS processes or halt undesired QS-regulated behavior such as virulence or biofilm formation (see reviews[41–43]). To modulate AHL QS processes, quorum quenching enzymes which degrade the autoinducers[44] can be expressed in engineered cells. A notable example includes human cells engineered to combat the pathogen *Pseudomonas aeruginosa* through a multiprong approach including controlled expression of quorum quenching lactonase and a biofilm disrupting glycoside hydrolase.[3] To modulate AI-2 QS, *E. coli* cells can be engineered to overexpress the AI-2 transporter and phosphorylation units.[36] Alternately, in some cases, virulence from a pathogen can be inhibited by designing cells that overexpress the QS molecule.[45,46]

4. Diversity of Scientific Expertise Broadens Applications of QS Systems

Metabolic engineers, molecular biologists, and now synthetic biologists have used engineered QS processes for tool development and engineered cell-cell communication, perhaps even predating the field of synthetic biology. Over the years, expertise from a variety of fields has broadened the possible applications of systems using rewired QS circuits.

Materials science has enabled development of a variety of capsules that can be used to contain or protect proteins and/or cells while still allowing diffusion of signaling molecules into and out of their shells. Gupta et al developed chitosan capsules containing AI-2 synthesizing "nanofactories."[47] These nanofactories consisted of a fusion protein capable of synthesizing AI-2 from the substrate, S-adenosyl-methoinine.[48] Capsules added to an *E. coli* culture were able to influence QS. Later, it was demonstrated that chitosan-alginate beads could be produced with bacterial "vacuum" cells engineered to rapidly consume AI-2.[49] The beads could quench QS in *E. coli* cultures. These capsules and beads allow engineered cells or nanofactories to influence QS processes with minimal contact and interference with the cells in the environments in which they are placed. Finally, artificial cells capable of cell-cell communication have been developed using phospholipid

vesicles.[4] That is, the basic premise for engineering QS communication is the modification of information transfer among bacteria, other cells, and even hosts. These examples show how engineered QS components and their microenvironments enable tailored information processing and even biological control.

While cells respond to a variety of inputs including physical cues such as temperature, pressure, light, etc., a preponderance of biological function is enabled by molecular cues, wherein "information" is contained both in the structure of molecules and their concentration, with the latter being altered by transport. When one expands the view of QS enabled information transfer, a natural extension is the interface with cues that are controlled by non-biological means such as light, magnetic fields, or electricity. One exciting emerging area of focus is the engineering of microbial systems for electronic surveillance and even control, or programmed biological function. By extension, electronic means that interface with QS signaling offer a pathway for coupling electronic systems with biological signaling. While the vast majority of QS signaling molecules have limited capability to exchange electrons with devices, the phenazines that, for example, are secreted by species such as *Pseudomonas*, are redox active and their oxidation state can be controlled both biologically and with simple electrodes.[50] Recognizing the interplay between redox biology, QS, and microdevices has led to such innovations as programmable biosynthetic flux through metabolic pathways assembled onto microdevices,[51] electronic control of gene expression,[52] and electronic transmission of information through complex regulatory circuits via a multiplexed electronic CRISPR.[53] By extension, electronic transmission of information via redox was recently shown to interact with co-cultures and mini consortia.[5]

5. QS in Cocultures, Consortia, and Microbiomes

An important and somewhat recent area of work is to use QS to control cell composition or phenotype in synthetic cocultures, microbial consortia, and microbiomes. Engineered cocultures can have several advantages over monocultures, including division of labor and modularity. QS provides one mechanism to regulate cocultures. For instance, Dinh and coworkers showed that in a naringenin producing coculture, QS circuits could be used to autonomously slow the growth rate of the population responsible for the first part of the pathway partway, presumably allowing the second population to complete naringenin synthesis in the batch coculture.[54] They demonstrated higher titers of naringenin could be achieved with the QS circuit. Synthetic cocultures may also be useful when deployed in native environments or as sensor systems. Stephens and coworkers developed an *E. coli* coculture system that when added to media with varying AI-2 levels, autonomously adjusts its culture composition based on the initial concentration of AI-2.[55] This was accomplished in part through autoinducer regulated cell growth rate, where HPr was upregulated in order to increase cell growth rate of one of the populations. In another example, redox molecules could be integrated with QS autoinducers to modulate gene expression in microbial cocultures in a context dependent manner.[56]

There is evidence that QS is important in natural microbial consortia and microbiomes, including in the GI tract. In these instances, engineered cells may be able to influence QS networks to improve health outcomes. Thompson et al demonstrated that *E. coli* engineered to overproduce AI-2 shifted the relative composition of Firmicutes and Bacteroides in the antibiotic treated mouse gut.[57] The mechanism behind this, however, is not clear. Indeed, much remains to be understood on the role of QS in microbial consortia and even in interkingdom signaling. Evidence exists of signaling between host and bacteria through host produced AI-2 mimics.[58] Even phage can potentially manipulate bacterial QS regulated processes.[59] Silpe et al recently demonstrated that a *Vibrio* phage encodes a receptor for a host produced autoinducer, and that binding of the phage receptor and host autoinducer starts the lysis program.[59] The authors demonstrated that engineering this phage could be used to program species specific kill switches.

It is increasingly clear that microbial consortia are important for human health,[60] plant health (which has implications for global food supply),[61,62] and even in breakdown of lignocellulosic biomass where consortia offer clues for efficient conversion of feedstocks into high energy products.[63,64] Studies suggest that QS plays a role in some of these cases, for instance in skin[65] and oral[66] microbiome health and in the plant rhizosphere.[67] In other cases, such as thermophilic bacterial consortia, little is known about whether QS is occurring, although studies indicate some thermophiles synthesize AI-2.[68] Additional fundamental science on the role of QS in these areas may lead to new applications, just as the newfound knowledge of QS mechanisms led to many of the engineered systems described above.

6. Summary, Outlook, and Personal Perspectives

In summary, engineers, inspired by QS processes that exist in nature, have engineered cell-cell communication through manipulation of QS pathways or assembly of QS components in new hosts. Increasing mechanistic understanding of QS circuits along with scientific advancements in a range of engineering disciplines has enabled the development of cells and devices for a breadth of applications.

Finally, we feel it is also important to provide personal perspectives on the role that Dr. Bassler has had on the field and on us, the coauthors of this work. To be specific, quorum sensing, its components, its architectural designs, its overarching functions, and its applications are now found in a great number of scientific fields that are highly diverse, spanning many disciplines. This is in large part due to the openness and intrinsic appreciation for scientific curiosity as well as the insistence of scientific rigor that are the fabric of Dr. Bassler.

One of the authors, Dr. Bentley (WEB), was invited by Dr. Bassler to attend one of the very first quorum sensing meetings of the American Society for Microbiology (as recounted below). The other author, Dr. Stephens (KS), graduated from Dr. Bentley's lab in 2020.

KS: When I joined Bill's lab, my career thus far consisted of completing my undergraduate degree in chemical engineering and a few years of work in two different industries. I had few professional female role models in these endeavors. To the best that I can recall, in four years of study, I took only two engineering, science, or math classes taught by women, and few women were part of the management team at either company. One of the first things I did after joining Bill's lab, on the advice of another student, was watch Dr. Bassler's recorded TedTalk on quorum sensing. As a first year graduate student, the talk was inspiring, and it was meaningful to see that a female professor was a leader in my new field. Throughout my PhD, I learned from and came to appreciate the scientific rigor in all the work coming out of Dr. Bassler's lab. Today, I work with cellulolytic and saccharolytic, non-model organisms. We still have much to learn about these species, but my training in quorum sensing and cell-cell communication encourages me to always be curious about how these microbes sense and respond to chemical cues in their environments. Over the past several years, many institutions and companies have made efforts to hire more women (I have witnessed all three academic departments that I have studied or worked at hire more women faculty), likely thanks in part to the tremendous scientific advancements made by Dr. Bassler and many other female scientists (including the editors of this issue!).

WEB: One of the very first quorum sensing meetings of the American Society for Microbiology was held in Banff, Alberta (2004). I was invited to attend by Dr. Bassler, who was meeting organizer and chair. To the best of my knowledge, I was the only engineer at the meeting. We had published in the *Journal of Bacteriology* the first microarray paper addressing genome-wide influence of AI-2[69] and using chemostat cultures, showed AI-2 production was a function of growth rate,[32] suggesting both a role as a signaling molecule as well as a metabolic byproduct. We had also conveyed that our focus was on understanding the role of AI-2 in elucidating metabolic function[35] as well as potential application.[70] That is, we wanted to first understand the molecular basis for cell-cell communication but also to take advantage of that understanding by using cell-cell communication in biomanufacturing processes like high cell density fermentations of *E. coli.* Bonnie's invitation to participate and her warm welcoming at the meeting (and indeed, that of the entire community) played a pivotal role in our continued interest. I'd also venture to say not just my own. Note the first author of all these papers, Matt DeLisa, is now an endowed professor at my own Alma Mater – ironically the same institution of Winans, Fuqua, and Greenberg in '94. As a chemical and now bioengineer, I have participated in a number of scientific "subfields" over the years, and observed that a subfield can grow or shrink based on the willingness of its participants to appreciate alternative opinions, embrace seemingly tangential observations, and at the same time insist on the importance of scientific rigor. Throughout the last nearly two decades, I've interacted with Dr. Bassler and without exception, enjoyed the openness, scientific insight, and friendship that has become a hallmark of her career.

Indeed, within the cell-cell communication community in the late '90s and early '2000's, there was active discussion regarding the role of AI-2 as either a signaling molecule or a metabolic byproduct. The tenor of the discussion, however, revolved around fundamental understanding, not right and wrong. Thanks to Bonnie, and the wise and helpful advice of Dr. Robert Kadner, who as an editor of the *Journal of Bacteriology* took the time to show this engineer how to justify that an experimental biological observation could stand within science as new knowledge, our laboratory has built on these molecular underpinnings, established new collaborations, and contributed to the intellectual merits of cell-cell communication – perhaps we have even had an impact on engineering practice.

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Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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