Chapter

Repurposing *E. coli* by Engineering Quorum Sensing and Redox Genetic Circuits

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Abstract

Because cells have the extraordinary ability to sense and respond to even subtle environmental changes by intricately regulating their gene expression patterns, their behaviors can be intentionally "tuned" by altering the state of their environments in a prescribed or rational manner. Rational control of both external and internal molecular stimuli provides a basis for many biotechnological applications including the expression of foreign protein products. This is done by coordinately controlling product synthesis while retaining the cell in a productive state. Quorum sensing (QS), a molecular signaling modality that mediates cell-cell communication, autonomously facilitates both inter- and intra-species gene regulation. This process can be rewired to enable autonomously actuated, but molecularly programmed, genetic control. Recently, even electrical signals, which have long been used to control the most sophisticated of man-made devices, are now employed to alter cell signaling processes enabling computer programmed behavior, particularly in cells suitably engineered to accommodate electrical signals. By minimally engineering these genetic circuits, new applications have emerged for the repurposing of *Escherichia coli*, from creating innovative sensor concepts to stimulating the emerging field of electrogenetics.

Keywords: protein expression, quorum sensing, autonomous induction, cell-cell communication, redox signaling, electro-induction, synthetic biology

1. Introduction

Controlling the processes or functioning of biological systems has profound implications in biotechnological and other applications [1]. By controlling gene expression, cell behavior and responses to environmental cues can, in turn, be regulated. Ever since the dawn of biotechnology, scientists have been searching for new and better methods to specifically modulate gene expression. Biological systems, however, possess the ability to sense and respond to internal or environmental changes through tuning their own genetic networks. For example, they are capable of detecting metabolic stress during foreign protein expression, and in response, express genes that brake or facilitate the process. Cells are also able to receive signaling molecules from their 'neighbors', to then begin functioning as a 'collective' or population by activating certain genetic regulons. In this chapter, gene-regulating technologies of prokaryotes are discussed that intentionally alter the intracellular landscape for protein expression

as well as the extracellular microenvironmental state in the vicinity of 'designer' production strains in order to program gene expression and behavior. These techniques incorporate the understanding of cell metabolism and the transcriptome, cell-cell communication (previously reviewed by [2, 3]), and biological redox reactions (previously reviewed by [4]). This chapter will mainly focus on recent advances in how actuation of genes is accomplished in *Escherichia coli* through methods that require only minimal genetic rewiring and the technologies developed on such platforms, for instance those of biosensors and bioelectric devices.

2. Optimizing protein expression: rational control of cell condition

There is no doubt that among the myriads of systems available for heterologous protein expression, the Gram-negative bacterium *Escherichia coli* remains one of the most popular owing to its relative simplicity, its inexpensive and fast high-density cultivation, its well-known genetics, and the large number of cloning vectors and mutant host strains that are commonly available. Though not every gene can be efficiently and fully expressed in this system, much progress has been made to improve the performance and versatility of this workhorse microbe. One of the most sought after outcomes is the overexpression of high quality target proteins, however difficulties such as stimulated protease activity and reduced growth rate, as pointed out decades ago, often arise accompanying overexpression [5–7]. In this section, a brief review of the general background pertaining to *E. coli* protein overexpression is presented.

2.1 Reducing bottlenecks: protease activity

The reduction in growth rate is particularly problematic, not only does it contribute to segregational plasmid instability, but severe growth rate perturbations at the onset of induced foreign protein synthesis have been shown to inhibit further expression of the desired protein [8]. Therefore, high levels of foreign protein expression are often unsustainable. Moreover, increased protease activity upon induction and overexpression of foreign protein generally leads to increased proteolysis, as described elsewhere [9–11]. These protease activities with uncharacterized specificity can be considered detrimental to the stability of the recombinant protein. Inefficient cell metabolism during overexpression, as indicated by acetate secretion of host cells, also results in lower protein expression [12]. These cell responses can greatly diminish the genetically-focused efforts to maximize both the final yield and concentration of recombinant proteins by increasing gene expression. In attempting to overcome these hurdles, cell dynamics during induced expression of chloramphenicol acetyl-transferase (CAT) expression have been examined and mathematically modeled in [13], suggesting that induction with an optimized amount of inducer (IPTG) at the onset of stationary phase can avoid growth rate suppression and achieve high expression. However, stimulated protease activity can be still observed. Intracellular proteases of recombinant *E. coli* have been differentiated by proteolytic activity and molecular weight and further characterized during the time course of protein overexpression [14]. Enhanced protease activity can respond quickly to induction, quicker than even the accumulation of the recombinant protein itself. To elicit and identify the proteases, transcriptional profiles of *E. coli* under stress of overexpression have been be mapped [15, 16]. Molecular chaperones (*groEL, ibpA*), lysis gene *mltB* and other DNA damage/bacteriophage associated genes (*recA*, *alpA*, *uvrB*) are all observed to be up-regulated along with proteases like *degP* and *ftsH*. It is also reported that cytoplasmic overexpression results in increased activity and expression of an outer membrane protease

OmpT [17]. With this understanding, "cell-conditioning" by adding dithiothreitol (DTT) to alter the levels of the aforementioned host cell proteins prior to product (e.g., CAT) overexpression is capable of placing the cell in a particularly productive state, the result being a doubling of product level [15]. Other methods such as RNA interference (RNAi), and more recent CRISPR technologies can be exploited to downregulate bottlenecks, such as proteases, while ensuring maximal expression of the desired genes. These methods can be targeted to specific genes or even entire regulons, depending on the applied stress and the desired effects.

2.2 Reducing bottlenecks: transcription factors

Levels of the global heat shock transcription factor, σ^{32}, for example, have been shown to increase rapidly during stress, including the stress associated with heterologous protein overexpression [18–23]. Indeed, a variety of cellular stresses induce the σ^{32}-mediated stress response, including both ethanol and heat shock [19–22, 24]. While σ^{32} accumulation could be mediated by control of transcription and translation, its accumulation following production of recombinant protein is mainly due to an altering of its otherwise chaperone-sequestered state [19, 25]. To facilitate protein expression in recombinant *E. coli*, many have posited that simultaneous downregulation of global regulators (such as σ^{32}) could simultaneously reduce the level of negative bottlenecks, such as the σ^{32}-activated proteases. Noting that σ^{32}-mutation is lethal at elevated temperatures [25, 26], methods such as RNAi were shown to transiently downregulate the σ^{32} stress response *in vivo* and these proved to be immensely advantageous. That is, using plasmids constructed with an antisense fragment of the σ^{32} gene, an early study showed that this successfully downregulated the expression of σ^{32} during the production of organophosphorus hydrolase (OPH), resulting increase specific OPH activity by six-fold compared to non-antisense-producing cultures [27].

2.3 Reducing bottlenecks: perspectives

Indeed, there have been countless studies demonstrating techniques to enhance the production of protein over the past 40+ years since recombinant DNA technology was first introduced. Besides choosing the right amount and type of inducer, optimal fermentation conditions have been developed to alleviate the reduction of growth rate during overexpression and enhance yield. Increasing stability of the protein product can also overcome the increased protease activity, this in addition to downregulation of protease-specific regulators. On top of the examples described above, an excellent review by Makrides [28] and a more recent review by Rosano [29] have discussed the various niches within which one can dig deeper in order to achieve higher yield and activity of the desired recombinant protein product.

We note that the majority of these methodologies have targeted either cell-based genetic regulatory structures, the sequence space and alterations of the protein of interest, or the operating policies of the reactors used to cultivate the overproducing cells. These cells, in turn, have typically been monocultures of an optimized host. Rarely have methodologies appeared in which collectives of cells, either monocultures or controlled co-cultures or consortia, and the exogenous signaling thereof are used to produce products such as recombinant proteins. Particularly useful when the engineering of a particular host overburdens its natural regulatory circuitry, cell consortia or collectives provide an interesting alternative. Co-culture and small consortia concepts have recently emerged. Moreover, new methodologies for orthogonal stimulation of genetic circuits can minimize pleiotropic or off-target effects normally accompanying more common chemical inducers. In the sections

that follow, we describe efforts to minimally alter the native bacterial signaling processes of quorum sensing and oxidative stress to repurpose *E. coli* for application in new platforms.

3. Decipher the bacterial dialog: quorum sensing

Gene expression in bacteria can be regulated by a wide array of intra- and extracellular cues. On top of the common chemical inducers that are most often introduced manually to initiate protein overexpression, bacteria are actually capable of producing their own extracellular signals for intercellular communication. The term "quorum sensing (QS)" was coined by EP Greenberg and colleagues decades ago, to describe the phenomena where the secretion and perception of small signaling molecules are transduced to coordinate behavior of a minimal unit (quorum) of microorganisms. Since then, there's been an explosion in understanding how bacteria communicate with themselves. In this section, well-characterized quorum-sensing systems and types of signals, receptors, mechanisms of signal transduction, and target outputs of each system are introduced. In addition, since quorum sensing in many bacteria is also shown to control gene expression in a global manner, several regulons will be introduced, again with the focus on *E. coli* and their potential application. Lastly, beyond controlling gene expression on a global scale, quorum sensing allows bacteria to communicate within and between species. Common pathways and inducers of interspecies communication will be introduced, and we will highlight some of the many applications built upon this ability to communicate not only between species, but also between kingdoms and non-biological substances. That is, by introducing QS phenomena, we develop its potential for keying protein expression via genetic or other means to cue its signaling processes.

3.1 Quorum sensing and its networks

Quorum sensing bacteria produce and release chemical signal molecules termed autoinducers, whose external concentration increases as a function of increasing cell-population density. Once the bacteria detect that autoinducers have reached a minimal threshold level of stimulatory concentration, they will respond by altering their gene expression and behavior. Autoinducers 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.

3.1.1 LuxIR system of V. fischeri

Quorum sensing mechanisms vary from species to species, and hence here we introduce the first-described QS system of the bioluminescent marine bacterium *Vibrio fischeri* as a paradigm for most systems in Gram-negative bacteria [30]. Relevant differences for each organism will be provided as necessary, yet an excellent review by Waters [31] has described most known systems in detail. *V. fischeri* infects higher order organisms, such as luminescent Hawaiian squid *Euprymna scolopes*, within its light organ is completely occupied by the bacterium. When confined, the bacterial population density can reach up to 10^{11} cells per ml and at that point luminescence genes are expressed through a QS mechanism. The luminescence shed by the bacterial consortium can be used, presumably for counterillumination to mask the squid's shadow so that it avoids predation.

Figure 1 illustrates the QS system of *V. fischeri*. Protein LuxI and LuxR control expression of the luciferase operon (*luxICDABE*) required for luminescence

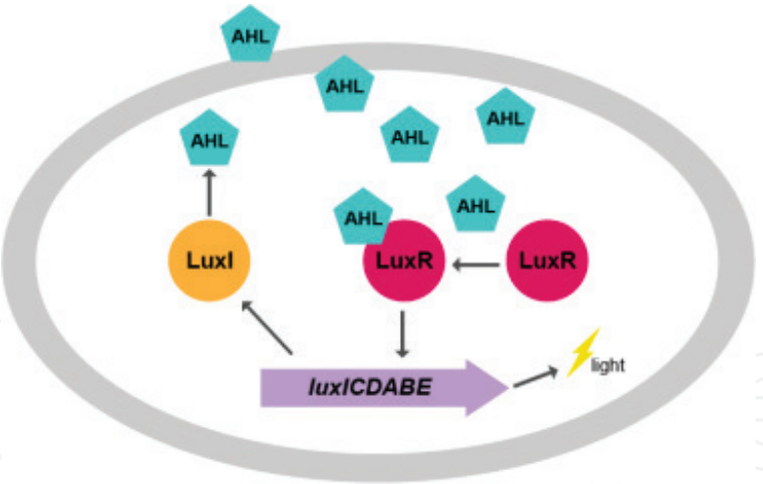

Figure 1.
Quorum sensing in Vibrio fischeri green pentagons denote AHL autoinducer that LuxI produces (3OC6-homoserine lactone). Transcriptional regulator, LuxR, modulates expression of AHL synthase, LuxI, and the lux operon, leading to luciferase-mediated light emission.

production. *LuxI* encodes for an autoinducer synthase that produces the acyl-homoserine lactone (AHL) autoinducer 3OC6-homoserine lactone. Following its production, the AHL will begin to accumulate - its concentration increasing as the cell density increases. Upon reaching a critical level, LuxR the cytoplasmic autoinducer 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 will be flooded with AHL which, in turn, switches all bacteria nearby to the QS active, light-producing mode [32]. The system observed in other *Vibrio* species is more complex, with additional sensing and phosphorylation components in the upstream of *luxR* [33]. In addition, small RNA (sRNA) have been shown to play a vital role in regulating the quorum circuits of *Vibrio harveyi* and *Vibrio cholerae* [34]. These LuxRI-type systems are mostly used for intraspecies communication, as extreme specificity exists between LuxR proteins and their cognate AHL autoinducer ligands.

3.1.2 LuxS/AI-2 system of E. coli

While some of the *Vibrio* QS components are present in *E. coli* (and *Salmonella* strains), the QS system of both species has been found to be distinctively different than that of the *Vibrio*. Several interspecies signaling systems have been identified: those mediated by LuxR homolog SdiA; the LuxS/autoinducer 2 (AI-2) system; an AI-3 system; and a signaling system mediated by indole [35]. Remarkably, the LuxS/AI-2 system possesses the unique feature of endowing cell-population-dependent behavior while interacting with central metabolism through the intracellular activated methyl cycle. LuxS intervenes in central metabolism by functioning in the pathway for metabolism of *S*-adenosylmethionine (SAM), the major cellular methyl donor. Transfer of the methyl moiety to various substrates produces the toxic by-product *S*-adenosylhomocysteine (SAH); while LuxS-containing bacteria have two enzymes (Pfs and LuxS) acting sequentially to convert SAH to adenine, homocysteine, and the signaling molecule DPD [31]. Together, LuxS/AI-2 system has the potential to regulate both gene expression and the cell fitness.

The *luxS* gene, which has a wide range of functions between numerous species, is responsible for AI-2 synthesis in QS networks. However, it was noted that the *luxS* transcriptional profile was reportedly unsynchronized with the accumulation profile of extracellular AI-2 in bacterial supernatants. Confounding its interpretation,

researchers turned toward the signal recognition motif. Thus, another component of the system: the *luxS*-regulated (Lsr) transporter that intakes the extracellular AI-2 was later discovered to be the reason behind the decrease in extracellular AI-2, and not LuxS protein, during stationary phase. As a part of the *lsr* operon, this ATP-binding cassette (ABC) transporter is regulated by both cyclic AMP/cyclic AMP receptor protein and LsrK/LsrR proteins that are transcribed in its own *lsrRK* operon located upstream of *lsr* [36]. The fact that AI-2 intake requires a separate transporter (LsrACDB) is backed up by [37]. Comparing to AI-1 (AHL, 3OC6-homoserine lactone), AI-2 (4,5-dihydroxy-2,3-pentanedione, DPD) is found to be less membrane active and does not intercalate into the bacterial membrane. After modification with carbon chains, products (especially heptyl AI-2) display strong surface activity. These results indicate that AI-2, a more hydrophilic entity, shows less affinity to lipids and thus requires a transportation system. **Figure 2** provides a schematic illustration of the *lsr* circuit comprising of *lsrACDB* (encoding the Lsr transporter), *lsrR* (encodes the transcriptional repressor), *lsrK* (encodes the AI-2 kinase), and *lsrFG* (encodes phosphorylated AI-2 (AI-2P) degradation enzymes) which are all directly regulated by AI-2. A recent mathematical model of this system was provided by Graff and Bentley [38], which helps to discriminate among hypothetical Lsr regulatory mechanisms and points to the importance of repressor LsrR dimer formation and binding on genetic regulation. Desynchronization of Lsr QS system, unlike the LuxIR system where its topology only consists of positive feedback, can display bimodal Lsr signaling and fractional induction. This phenomenon has been both observed in experiments and was also simulated with a mathematical model [39].

3.2 Global quorum sensing regulons

3.2.1 Global genetic regulation of LuxIR and AI-2/Lsr systems

The dawn of genomic profiling has unveiled that quorum sensing, in many bacteria, controls gene expression in a global manner. QS-mutants of *S. pneumoniae*

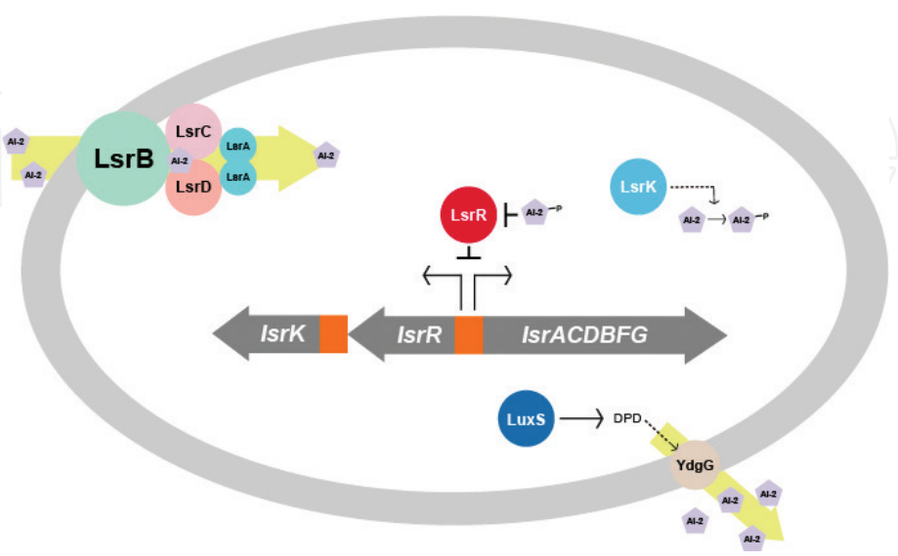

Figure 2.
Regulatory mechanisms of the lsr/AI-2 circuit in E. coli. AI-2 is imported by the Lsr transporter (LsrACDB) and in turn, is processed by LsrK, transforming to its phosphorylated form (AI-2P). As AI-2P binds LsrR, it relieves the repression of LsrR on the Lsr genes and accelerates AI-2 intake. LuxS produces DPD, the precursor of AI-2. The autoinducer is then transported out of the bacteria by YdgG (TqsA), a putative transporter belonging to the exporter superfamily [40].

and related *Streptococci* show defects in multiple pathways, including biofilm formation, acid tolerance, bacteriocin production, and virulence [31]. *E. coli*, too, has been reported to elicit broad QS activities. For example, the quantity and architecture of biofilms are regulated by *lsrR/K* through motility QS regulator (MqsR, B3022), as well as the generation of several small RNAs [36, 41]. Together, these and other reports suggest that QS systems control many aspects of the whole genome rather than just one key gene locus. Further evidence that quorum sensing coordinates the control of a large subset of genes comes from 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 [42–44].

Interestingly, AI-2 synthesis and signaling levels are linked to the accumulation of protein product expressed from plasmid-encoded genes [44]. This suggests that recombinant *E. coli* are able to communicate the burden of overexpressing heterologous protein through AI-2 QS pathways. Most recently, the sugar metabolism of *E. coli* was found to be directly connected to the LuxS/AI-2 QS system. That is, HPr, a phosphocarrier protein central to the sugar phosphotransferase system, was recently reported to copurify with LsrK such that the activity of LsrK was inhibited when bound to HPr [45]. In sum, these finding shed new light on how bacteria respond to changing nutrient levels on a population scale. The intentional manipulation of the QS signaling processes, therefore, has become an interesting target for heterologous gene expression in *E. coli* among many other applications [46].

3.2.2 Applications

De novo engineering of gene circuits inside cells is proven to be difficult, in large part due to connectivity to non-targeted pathways and genes [47, 48]. QS regulons, coupling intraspecies communication and global genome regulation, can serve as excellent platforms for many technologies to be built upon, particularly if one understands the regulatory "reach" of the genetic circuits. Attempting to eliminate the variation in phenotype between cells, You et al. coupled gene expression to cell survival and death using the LuxIR QS system [49]. With the 'population control' gene circuit, they successfully regulated the density of an *E. coli* population autonomously and were able to program the dynamics of an entire population despite behavioral variability between individual cells. Based on the same LuxIR system of *V. fischeri* and the QS system of *Bacillus thuringiensis*, a synchronized genetic clock was engineered [50]. This novel gene network with global intercellular coupling can generate synchronized oscillations in a growing population of cells. In biology, synchronized oscillation holds the same importance as in physics and engineering, where it governs many fundamental physiological processes such as cardiac function and circadian rhythm [51]. These studies have set the stage for future development of using microbes as macroscopic biosensors with oscillatory output, as the colony-level synchronized oscillation could diminish single-cell variability in most synthetic gene networks and increase the sensitivity and robustness of response to external signals.

On top of employing the LuxIR system as a platform for innovative genetic and population regulators, intentional rewiring of *E. coli*'s native QS networks can also benefit biotechnological applications. For example, in [52], autonomous induction of recombinant proteins is realized through minimal rewiring of the AI-2/Lsr system. Since the QS network is capable of 'reporting' the metabolic state of a bacterial population and the metabolic burden is self-indicated by this network [44], Tsao et al. made it possible to achieve metabolically-balanced coordination of the entire culture for a user-specified purpose through minimal rewiring of the QS network

and signal amplification by the T7 RNA polymerase [53, 54]. This study demonstrated one cell population was able to guide protein synthesis process of another by guiding intraspecies communication. Moreover, it was reported in [55] that by simply adding conditioned medium (containing a high amount of AI-2) during recombinant protein induction, one can double the yield of active product. Also, by altering the coincident *luxS* expression to control the AI-2 concentration while also inducing heterologous protein expression, they found an optimal condition where protein yield is dramatically increased. The authors further elucidated the mechanism behind this phenomenon: chaperone GroEL was shown to be coincidentally upregulated post-transcriptionally by AI-2. Because of its native role as a stabilizer of heterologous protein and its role in folding, the upregulation of GroEL might be the reason behind the higher product yield.

More endeavors have been made [56] to increase protein yield in this autonomous system through a different approach. With the same intention in mind [49, 50], a new study showed that reduced heterogeneity between independent cells could be achieved by inserting an enhanced feedback loop to the *E. coli*'s native AI-2 QS system. Upon activation of the engineered system, not only does the foreign pET plasmid concurrently express more sfGFP signal, but it also transcribed more LsrACDB and LsrK than the native *lsr* operon [57]. This overexpression resulted in increased uptake of AI-2, leading to amplified system response and minimized heterogeneity. Heterogeneity, on the other hand, could also be leveraged. In [58], quantized *E. coli* quorums were intentionally assembled through independent engineering of the AI-2 transduction cascade increasing the sensitivity of detector cells. Upon encountering a particular AI-2 level, a discretized sub-population of cells emerge with the desired phenotype. This sensitive, robust detection process could pave the way for future cell-based biosensors for AI-2 and subsequent programmed cell function.

That is, in [59] and as shown in **Figure 3**, *E. coli* were modified to enable programmed motility, sensing and actuation based on the density of user-selected features on nearby surfaces. These 'smart' bacteria can then express marker proteins to indicate phenotypic response based on calculated feature density displayed on the surfaces of nearby eukaryotic cells. Specifically, the AI-2/Lsr signaling pathway was rewired an introduced onto the eukaryotic cells as a 'nanofactory' to direct *E. coli* to swim toward a cancer cell line (SCCHN), where they then initiated synthesis of a drug surrogate based on a threshold density of epidermal growth factor receptor (EGFR). This novel technology represented a new type of targeted drug synthesis and delivery and a new area-based switch that could serve multiple purposes within in the field of synthetic biology.

3.3 Interspecies communication

3.3.1 Universal autoinducer AI-2

Beyond controlling genetic expression on a global scale, quorum sensing allows bacteria to communicate within and between species. This notion arose with the study and discovery of the aforementioned autoinducer AI-2. Derived from SAM as a part of bacterial 1-carbon metabolism, AI-2 is a general term for a family of cyclic furanones utilized in interspecies communication [60]. In LuxS-containing bacteria, SAM is converted into SAH and then broken down by enzymes Pfs and LuxS sequentially into signaling molecule DPD and other byproducts. Due to the high reactivity of DPD, many distinct but related products could be recognized by different bacterial species as AI-2. Though it is postulated that small molecules of similar structure as AI-2 could serve as potential antagonists that halt the bacterial conversation, only a handful are found (compared to a large number of AI-1 inhibitors). In [61], C-1

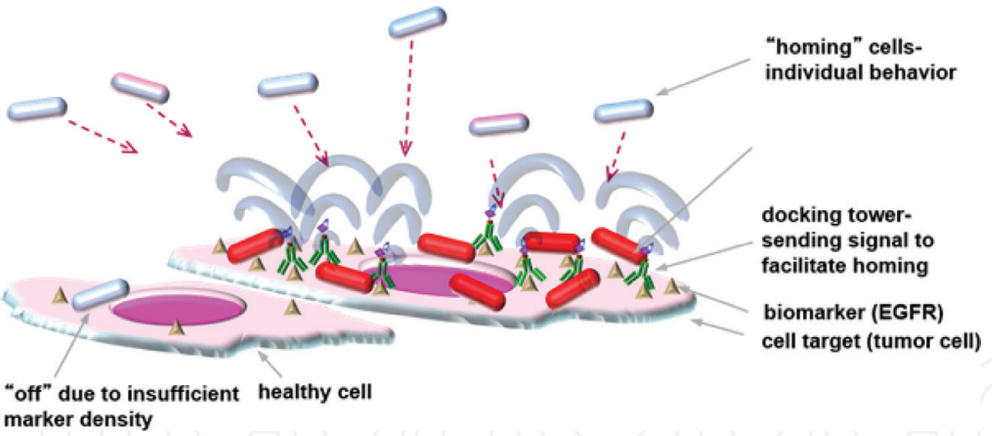

Figure 3.
Biological nanofactories that synthesize AI-2 are targeted to EGFR on the surface of SCCHN cells. AI-2 is emitted from the cell surface and recognized by reprogrammed bacteria, which swim to the site of signal generation and decide, based on AI-2 level (proportional to the EGFR surface density), whether to initiate gene expression (adapted from Wu et al. [59]).

alkyl analogs of AI-2 that quench QS responses in multiple bacterial species simultaneously were developed and synthesized. Interestingly, addition of a single carbon to the C1-alkyl chain of the analog plays a critical role in determining the effect on quenching the QS response. This analog, isobutyl-DPD, was later used to inhibit maturation of *E. coli* biofilms [62]. An expanded and diverse array of AI-2 analogs, including aromatic and cyclic C1-alkyl analogs are synthesized in [63]. Some were identified as species-specific QS disruptors for *E. coli* and *Salmonella typhimurium*, and so were QS quenchers for *Pseudomonas aeruginosa*. Remarkably, these synthetic analogs selectively antagonized quorum sensing among individual bacterial strains within a physiologically relevant polymicrobial culture.

AI-2 is also one of the several signals used by marine bacteria *V. harveyi*. Specifically, AI-2 encoding *luxS* has been found in roughly half of all sequenced bacterial genomes. AI-2 production has been verified in over 80 species, and AI-2 controls gene expression in a variety of bacteria. By using Local Modular Network Alignment Similarity Tool (LMNAST) to study gene order and generate homologous loci, the AI-2/Lsr system was reported to be phylogenetically more dispersed than the well-studied *lac* operon, while its distribution remained densest among gammaproteobacteria [64]. These findings together reinforced the hypothesis that bacteria use AI-2 to communicate between species [31, 65].

Interkingdom communication was also shown to be mediated by AI-2. In [66], transcriptomic effects of bacterial secretions from two nonpathogenic *E. coli* strains (BL21 and W3110) on the human colonic cell line HCT-8 were explored using RNA-Seq. Expression of inflammatory cytokine interleukin 8 (IL-8) in HCT-8 cells was found to respond to AI-2 with a pattern of rapid upregulation followed by a subsequent downregulation after 24 h. This discovery helps provide a deeper understanding of the relationship of microbiome and the host, which is of significant importance in maintaining human health.

3.3.2 Applications

This discovery suggests that AI-2 QS manipulation might find application in guiding human physiology and that 'smart' bacteria, those making heterologous proteins such as drugs or essential nutrients and that otherwise serve as decision makers, might find application in a variety of other fields. As an extension, Lentini et al. [67] engineered minimal 'artificial' cells capable of expressing AI-2 synthesizing fusion protein His_6-LuxS-Pfs-Tyr_5 (HLPT) [68] wherein newly synthesized

AI-2 was proven to induce luminescence in nearby cells, particularly an AI-2 reporter strain of *V. harveyi*. This not only demonstrates QS-mediate communication between cells and non-biological, artificial cell mimics, but presents a new technique to alter the complex networks of natural cells without tampering with the original genetic makeup.

Developing, silencing, or intervening with the communication between cells has revolutionized the way we control gene expression. In [69], communication between cells is developed further by modifying the biological 'nanofactories' proposed by LeDuc et al. [70] to trigger QS responses in the absence of autoinducers. They are self-assembled and comprised of four functional modules: a targeting module (an antibody), a material sensing module, an assembly module, and a synthesis module (fusion protein His_6-Protein G-LuxS-Pfs-Tyr_5, HGLPT, (**Figure 4**). Protein G (assembly module) allows the chimeric enzyme to attach to a targeting antibody *ex vivo*, and LuxS and Pfs together convert raw material, SAH, into autoinducer AI-2. The targeting antibody is proven to successfully attach onto targeted *S. typhimurium* in a mixed culture that also includes *E. coli*. Remarkably, this study built up interspecies 'conversation' between cells that do not usually communicate with each other. After *E. coli*-targeted nanofactories were added to non-QS *E. coli* to 'unmute' the null *E. coli*, the activated *E. coli* are co-cultured with reporter *luxS* null *S. typhimurium*. As the levels of activated *E. coli* increased, *S. typhimurium* begin to 'respond' as they received the AI-2 produced by activated *E. coli* and initiate the expression of their own reporter gene. Interkingdom communication between *E. coli* and human intestine epithelial (Caco-2) cells was also developed using this technique [71]. This tool may be very useful for interrogating and interpreting signaling events in human GI tract.

Perhaps next generation antimicrobials can be created by intercepting bacterial communication and creating 'smart' bacteria. Instead of targeting the viability of pathogenic strains, interruption of their communication is proposed, as it is hypothesized that there will be less selective pressure to develop resistance if instead one targets the mechanisms keyed to pathogenicity [72]. As a global autoinducer, 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 are all likely targets for inhibition, especially as there are many synthesized AI-2 analogs that are available for quorum quenching [61–63, 73]. In another case [74], probiotic *E. coli* were themselves, engineered to eliminate and prevent *P. aeruginosa* gut infection by reducing biofilm formation. However, it was the *P. aeruginosa*-secreted, species-specific autoinducer AHL ($3OC_{12}HSL$) secreted detected by the probiotic *E. coli* and served as the trigger the for the expression of an anti-biofilm enzyme dispersin B (DspB) and a *P. aeruginosa* toxin, pyocin.

In addition to potential for therapeutic synthesis and delivery, *E. coli* cells can be rewired to serve in networks that provide molecular information about their surroundings or as cell sensors or 'sentinels'. For example in [75], engineered *E. coli* sentinels are made to recognize and move toward hydrogen peroxide, a non-native chemoattractant and potential toxin. Similarly, commensal gastrointestinal strain *E. coli* Nissle 1917 are engineered to recognize gastrointestinal dysfunction biomarker nitric oxide (NO) [76]. These 'smart' bacterial sensors can generate strong fluorescent response upon NO recognition and may serve as simple diagnostic tool for diseases like Crohn's Disease and ulcerative colitis. In [77], nano-guided cell networks that serve as conveyors of molecular communication are developed (**Figure 5**). This system interprets molecular information by intercepting diverse molecular inputs, processes them autonomously through independent cell units within the system and refines output to include positive responders that are viewed via orthogonal, simple optical means. That is,

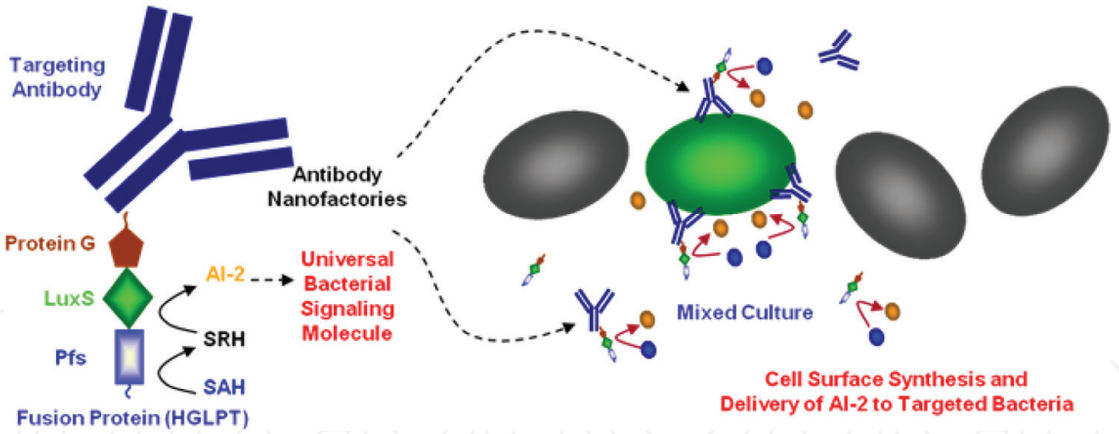

Figure 4.
Biological nanofactory induced interspecies communication. SAH (blue circle) is converted into AI-2 (yellow circle) by the nanofactory fusion protein anchored onto E. coli. AI-2 thus activated QS gene expression in reporter cells (adapted from Fernandes et al. [69]).

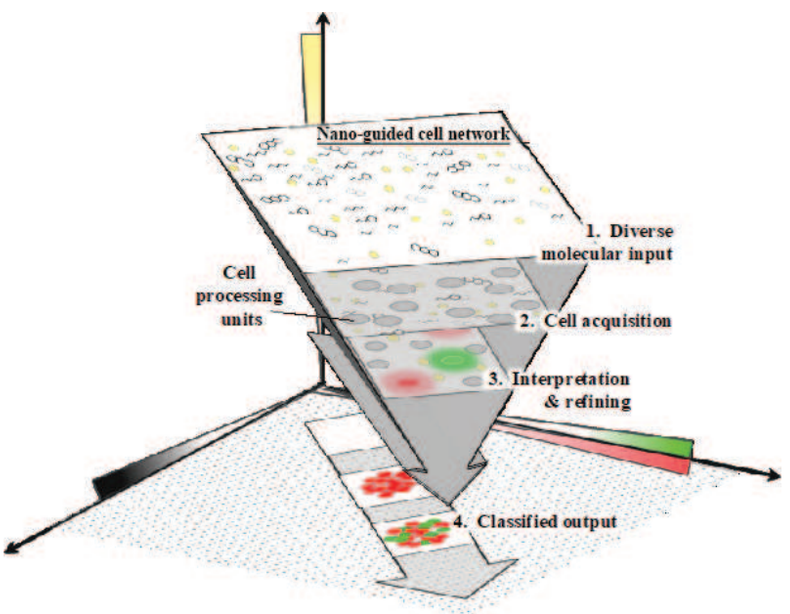

Figure 5.
Schematic of a cell population and nanomaterial-based network. This conceptual system describes cells and magnetic nanoparticle networks that intercept diverse molecular inputs, process them autonomously through independent cell units, and refines output to include positive responders that are viewed via visual classification (red or red and green, adapted from Terrell et al. [77]).

in the preceding sections we have described how engineering cells and the signaling processes that guide their behavior can be used to enhance the overall expression of proteins, but also that when coupled with more advanced functions, cells can serve as their own autonomous factories or surveyors of various microenvironments. A key to performing these functions in an optimal manner is the control of the signaling process, the signal itself, its positioning, its strength or frequency, and its recognition by the engineered cell.

4. Bridging the bio-electro interface: Redox signaling and electrogenetic systems

In addition to quorum sensing, bacteria use numerous other small chemical molecules to build up conversations between themselves and with the environment.

It is well known that redox reactions and redox based signaling pervade living cells and are extremely crucial to both anabolic and catabolic metabolism. Redox-based molecular systems, however, are also leveraged by bacteria for communication. Cells must detect a variety of oxidative stressors and quickly respond so as to avoid oxidative damage and maintain redox balance in order to survive. In this section, several redox signaling pathways will be introduced, yet emphasis will be on how redox signaling and electrochemistry help connect communication and information transfer between biological systems and electronic devices. In this way, redox molecules can serve as exogenous and electronically-programmed controllers of biological function.

4.1 Redox signaling in biological systems

In response to redox imbalance, new metabolic pathways are initiated, the repair or bypassing of damaged cellular components is coordinated and systems that protect the cell from further damage are induced. Throughout the years, many studies have revealed a vast repertoire of elegant solutions that have evolved to allow bacteria to sense and respond to different redox signals [78]. Below, two oxidative stress sensors, SoxR and OxyR, and their corresponding signaling pathway will be introduced. These systems are later shown to enable electrical control of gene expression in *E. coli*.

4.1.1 SoxR: [Fe-S]-cluster based, superoxide/nitric oxide stress sensor

The *E. coli* SoxRS system enhances the production of ~45 proteins in response to superoxide exposure, including those in detoxification (*sodA*, manganese superoxide dismutase), DNA repair (*nfo*, endonuclease IV), maintaining cellular reducing power (*zwf*, glucose-6-phosphate dehydrogenase) and central metabolism (*fumC*, superoxide-stable fumarase C and *acnA*, aconitase A). The *E. coli* SoxR protein exists as a homodimer that contains one [2Fe–2S] cluster per subunit. During aerobic growth, up to 95% of SoxR are held in the reduced ($[2Fe–2S]^{1+}$) state. Upon sensing conditions that promote the production of superoxide, SoxR is oxidized to ($[2Fe–2S]^{2+}$) clusters and it leaves the *SoxR/S* promoter region (*psoxRS*) to activate the expression of transcription factor SoxS. SoxS, unlike SoxR, when bound to *psoxRS* initiates the expression of the proteins listed above located downstream of the promoter [78, 79].

4.1.2 OxyR: thiol-based, peroxide stress sensor

The *E. coli* transcriptional activator, OxyR, is a member of the LysR family of transcriptional regulators. Although it is often cited as the model for bacterial redox sensors, the precise mechanism of thiol modification and the consequences for OxyR activity are the subject of ongoing controversy [80]. Like SoxR, OxyR acts as a repressor of *oxyS* RNA transcription in *E. coli*. Oxidation of cysteine residues in OxyR results in a dramatic secondary structure rearrangement, which leads to a change in the DNA-binding specificity of OxyR, recruitment of RNA polymerase to *OxyR/S* promoters, and the subsequent transcriptional activation of downstream genes such as *oxyS*. OxyS RNA, in turn, is a global oxidative stress regulator mediating the activation or repression of over 40 genes, including several detoxifying enzymes such as hydroperoxidase I (*katG*) and alkylhydroperoxide reductase (*ahpCF*) [75, 78]. Responses of *katG* and *ahpCF*, along with many genes in *SoxR/S* regulon (*sodA, zwf, fumC* and *acnA*) upon paraquat (superoxide ion regenerating redox reagent) insult have been revealed in [81].

4.2 Redox capacitor and bio-electrode interface communication

To probe bio-related redox reactions/signaling simply and readily, recently developed redox-capacitor films can serve as a bio-electrode interface. These are well-described and have been reviewed [82]. In brief, these electrochemical tools are capable of accepting, storing and donating electrons from mediators commonly used in electrochemistry and also in biology. Biofabricated from catechol and the polysaccharide chitosan, the former can be readily (and reversibly) oxidized. When catechol is oxidized, quinone is formed and it can be covalently grafted onto chitosan. In addition, chitosan can be easily 'electro-assembled' onto electrodes owing to its pH-responsive properties. That is, when a voltage is applied to an electrode submersed in an aqueous solution containing chitosan, the pH near the electrode can be controlled. When basic (above the pKa of chitosan, ~6.5), chitosan will form a hydrophobic network and assemble onto the electrode as a film or hydrogel, depending on the application of the electronic charge. When the catechol/quinone redox couple is integrated into the film, it can serve as a source or sink of electrons. Diffusible redox mediators can be added as they can exchange electrons ('charge/discharge') with the redox-active films. Common biology-related mediators include molecules such as ascorbate and NADH, which can charge and discharge the film. Pyocyanin, a toxin secreted by *P. aeruginosa*, is also found to be able to donate electrons to catechol-chitosan film (charging). This metabolite is noted because it, like many other mediators, can also undergo redox-cycling in the film to amplify outputs and facilitate detection of is host cell. It can similarly carry electrons from electrodes directly to proteins or cells near the electrode where such transfer of 'information' can control biological processes.

4.3 Electrical process modulation and gene induction

Many researchers have endeavored finding new ways to control cell processes. The use of optical means to regulate gene expression has garnered significant attention and resulted in an entire field of optogenetics [83]. Genetic switches that operate on optical signals (even small changes in wavelength or color) have been shown to be powerful exogenous controllers of cell function [84, 85]. More recently, researchers have turned to electronic devices to directly control biochemical reactions. In [86], a transistor-like device is engineered to control glucose metabolism of yeast (*S. cerevisiae*). Changes in gating voltage of the device are reported to bring about acceleration or deceleration of the depletion rate of glucose, and in turn the production rate of end-products (ATP and ethanol). Biofabrication and cell-based communication can also be enhanced through electrical control. In a nano-biosystem [87], electrical signals were used to assemble and tune an enzymatic pathway. The assembly comprised of electrodeposited chitosan film on top of a gold electrode, followed by the enzymatic and covalent grafting of a model enzyme HLPT [68] onto the chitosan scaffold. Through different electrical signals and with the help of diffusible redox mediators (pyocyanin), not only the amount of assembled enzymes but their activity was found to be tunable.

Even more recently, a synthetic, mammalian electro-genetic transcription circuit was created [88]. This was done by linking the electrochemical oxidation of ethanol to acetaldehyde, triggering an acetaldehyde-inducible gene expression circuit. While an indirect outcome of the applied voltage, this was the first study whereby specifically intended gene expression was induced by electronic means. A more direct methodology recently appeared [89] in which the engineered genetic circuit responds directly to the electrode-oxidized signal molecule, opening an entirely new modality for bioelectronic control (**Figure 6**). Again, pyocyanin was

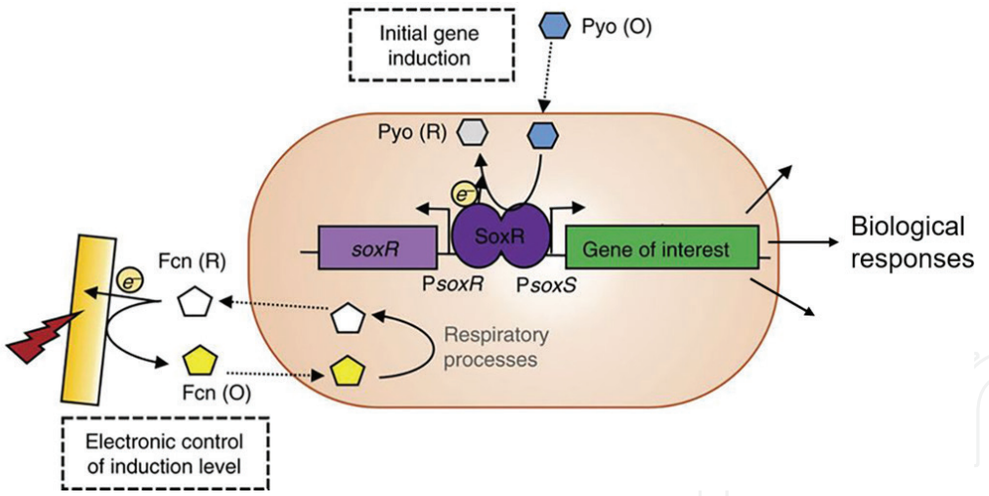

Figure 6.
Electrogenetic induction system scheme. Pyo (O) initiates gene induction and Fcn (R/O), through interactions with respiratory machinery, allows electronic control of induction level. Fcn (R/O), ferro/ferricyanide; Pyo, pyocyanin. Encircled 'e⁻'and arrows indicate electron movement (adapted from Tschirhart et al. [89]).

used in their system, it is responsible for translating electrical signals into a biochemical redox signal that, in turn, can be sensed by SoxR and in sequence, initiate the expression from *psoxS* promoter. Strikingly, gene expression controlled by this device is functionally reversible on relatively short time scales (30–45 min). It was also found to be quite robust, as oscillatory behaviors were shown over many cycles. Accordingly, both optogenetic and electrogenetic systems will require that an entirely new 'suite' of genetic elements be developed that respond to and coordinate these environmental cues. In the recent study, the expression of AHL-synthesizing enzyme LuxI was electronically actuated, resulting in electronic control of QS behavior of nearby cells. Analogously, motility regulator CheZ was also electronically stimulated demonstrating the electronic initiation of cell motility. This study is the first in which electronic signals guided engineered cells and those, in turn, guided others. While this chapter has focused on gene expression in *E. coli*, it also attempts to show how the simultaneous coordination of gene expression and of the host cells can result in interesting and new application areas.

5. Conclusion

Researchers in biotechnology are constantly seeking novel platforms or techniques from which to address problems: those that in a broad sense, have enhanced efficacy, while maintaining or intensifying specificity. In this chapter, innovative means that focus on controlling environmental cues to regulate gene expression are introduced. To optimize heterologous protein expression, methods seeking to repress stress responses and retain cells in a 'productive' state are carried out by carefully engineering host cells to respond to various cues that are either introduced exogenously or endogenously. QS systems have appeared that provide targets for controlling bacterial behavior. They are also shown to report on the prevailing metabolic state of a product-producing cell. Early methodologies such as RNAi, genetic mutation, product protein-directed evolution, all successful means to enhance yield, can be reexamined based on new understanding of how cells communicate with one another. That is, QS systems enable the rewiring of endogenous metabolism for the coordinated control of entire populations of cells. This ushers in a new way of viewing protein or product-producing cells as a cell 'collective' rather than as individual cells each identical to one another, responding to cues or inducers such as

IPTG for the controlled overexpression of heterologous proteins. QS systems enable autonomous global gene regulation based on cell density. That is, instead of direct interrogation and control of genetic circuits, QS-based cell-cell communication allows indirect gene regulation through self-secretion and uptake of small signaling molecules. Further, exogenous and orthogonal signals, such as those provided by optical and electrical means can be interfaced with cells, providing exquisite control of gene expression. Importantly, in host cells were synthetic components contribute minimal perturbation to native systems, exogenously signaled protein expression can be coupled with exogenously controlled cell behavior (e.g., swimming or decision making). Electrochemistry, along with the invention of redox capacitors, thusly opens a new niche for genetic induction. That is, by leveraging the ability of mediators to translate electrical signals into chemical cues, researchers can cue changes in environmental electrical state that, in turn, are capable of inducing gene expression. These innovative methods will no doubt continue to generate impactful applications in fields such as biotechnology and biosensing.

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Conflict of interest

We declare that we have no conflicts of interest associated with the submitted work.

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