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Abstract

Bacteria across many scales are involved in a dynamic process of information exchange to coordinate activity and community structure within large and diverse populations. The molecular components bacteria use to communicate have been discovered and characterized, and recent efforts have begun to understand the potential for bacterial signal exchange to gather information from the environment and coordinate collective behaviors. Such computations made by bacteria to coordinate the action of a population of cells in response to information gathered by a multitude of inputs is a form of collective intelligence. These computations must be robust to fluctuations in both biological, chemical, and physical parameters as well as to operate with energetic efficiency. Given these constraints, what are the limits of computation by bacterial populations and what strategies have evolved to ensure bacterial communities efficiently work together? Here the current understanding of information exchange and collective decision making that occur in microbial populations will be reviewed. Looking toward the future, we consider how a deeper understanding of bacterial computation will inform future direction in microbiology, biotechnology, and biophysics.

1. The computational potential of microbial communities

Bacteria interact with their environment in astonishing ways and to perform complex, coordinated tasks. We now know that bacterial cells have developed mechanisms to monitor and respond to changes in physical and chemical conditions as well as communicate with each other to work as multicellular collectives. Coordination of such behavior is an example of collective or swarm intelligence, as groups of cells gather information to collectively compute a response.

Recently the ability to gather and respond to information is gaining attention as the essential feature of living systems. Biological molecules change structure as they interact with other molecules and these changes transmit information. Bacteria receive and process information from their extracellular environment in order to compute an adequate response or 'make a decision' [1]. What does it mean for bacteria to compute? By 'compute' we mean a process by which groups or individuals gather and use information to change the composition, spatial structure, or activity of a community of cells. A population of multiple species or strains of cells and their interactions through the exchange and detection of small molecules constitutes a 'network'. The coordinated behaviors that emerge from bacterial networks can be viewed as microbial intelligence. These behaviors confer to the population metabolic and informative benefits that improve fitness. Here we review recent work examining how signal exchange is used to gather information from the local environment to compute collective responses. We then examine how such bacterial computations are efficient means of information processing by bacterial collectives.

Information is gathered by microbes using molecular sensors. Bacterial cells express a variety of sensors, many in the form of two-component systems, although one-component systems are widely distributed among prokaryotes [2]. Two-component systems are composed of a sensor embedded on the membrane that detects a variety of chemical and physical changes in the environment, and a response regulator that modulates patterns of gene expression based on such stimuli [3]. The ability of bacterial species to utilize a collection of two-component

systems, and several other signal transduction pathways, to sense and respond to a variety of external inputs has even been used to quantify bacterial IQ [4]. In addition to being able to passively detect changes in the molecular environment around each cell, bacteria actively probe their immediate surroundings and communicate with each other through the exchange of molecular signals.

A well-known example of this active process of gathering information from the environment to regulate cellular behavior is through a process known as quorum sensing. Quorum sensing is the ability to emit chemical signals, called autoinducers, and respond to high concentrations of these signals by enacting large changes in gene expression profiles [5–9]. Despite decades of work uncovering and characterizing molecular components related to quorum sensing, debate remains over what specific types of information and collective benefits bacteria gain in the process of quorum sensing [10].

Historically quorum sensing has been described as a mechanism to monitor population size, as a large population of signal producing cells leads to a high concentration of signals, as depicted in figure 1(a). Quorum sensing-regulated gene expression usually occurs as signal concentration exceeds a threshold. As the density of well-mixed populations exceeds around 10^7 cells/mL [11], a switch-like activation [12], typically results in major changes in gene expression [13, 14]. Genes differentially expressed when quorum sensing is activated often are related to high cell density behaviors and cooperativity within large populations [15]. Quorum sensing-regulated genes control biofilm formation and horizontal gene transfer [14, 16], and the expression of exoenzymes for harvesting shared, public nutrients [17, 18]. Given the fitness advantage of cooperation, quorum sensing mechanisms are common to many bacterial species [19].

Over the years, the simple perspective of quorum sensing as a way to measure population density has evolved to encompass more sophisticated views of the computational potential of bacterial signal exchange. Here we highlight two such computations that bacteria perform as the result of quorum sensing, efficiency sensing and monitoring bacterial community composition.

2. Computation of the spatial environment

2.1. Computing the efficiency of signal exchange

The ability of quorum sensing signals to probe the propensity for released biomolecules to act locally has been referred to as efficiency sensing [20]. In this reframing, bacterial quorum sensing is a measurement of the local accumulation and reabsorption of any externally released biomolecule, see figure 1(b). This perspective focuses on the importance of mass transfer and the spatial distribution of cells within populations that are not well mixed.

An example of efficiency sensing is quorum sensing in confined spaces. Confinement of even small numbers of cells restricts loss of released autoinducers due to diffusion, as would occur in large volumes. When *Pseudomonas aeruginosa* cells were confined in microfluidic droplets, quorum sensing was achieved even in populations with only a single cell, see figure 1(c) [21]. The ability of cells to detect confinement via quorum sensing has also been observed in *Staphylococcus aureus*, both in microfluidic experimental systems and in the realistic context of cells engulfed by phagosomes [22, 23].

Quorum sensing in the presence of flow is another context in which cells could calculate the efficiency to retain released molecules [24–26]. Emge *et al* [24], studied the quorum sensing of wild type *P. aeruginosa* and an engineered *Escherichia coli* carrying the same quorum sensing gene circuit under controlled flows. High flow rates suppressed the expression of quorum sensing-regulated genes by sweeping away signal that would otherwise be taken up by the cells, despite cells being located in a high cell density environment, as shown in figure 1(d). Cells are keenly attuned to their extracellular environment through quorum sensing.

As demonstrated in these examples, bacteria use cell-to-cell signaling pathways to gain information about the local environment. A mathematical model by Cornforth *et al* [27], also demonstrated how quorum sensing systems with multiple autoinducers with distinct half-lives could enable bacteria to infer both their density and mass-transfer environment at the same time. In this model, the production of two autoinducers by *P. aeruginosa* enabled distinction between four possible combinations of high and low mass transfer and cell density.

2.2. Pattern formation in response to spatial structure

Pattern formation is symptomatic of bacteria engaged in coordinated, regulated behavior. Quorum sensing plays an important role in bacterial self-sorting into specialized, physically localized domains that in some cases resemble multi-cellular organisms [28]. *Myxococcus xanthus*, for example, leverages quorum sensing to initiate the formation of elaborate, self-organized fruiting bodies [29].

The distribution of cells in space strongly influences cellular communication. The dynamics of diffusion set the spatial and temporal scale over which cells interact with neighbors. Space also introduces additional nonlinearity to the interactions within biological networks [30]. In well-mixed conditions, all cells see the same chemical environment, within small molecular fluctuations. The response of cells within these populations is approximately uniform. Contrast this behavior with that of cells in a biofilm, where cell–cell communication is spatially dependent and

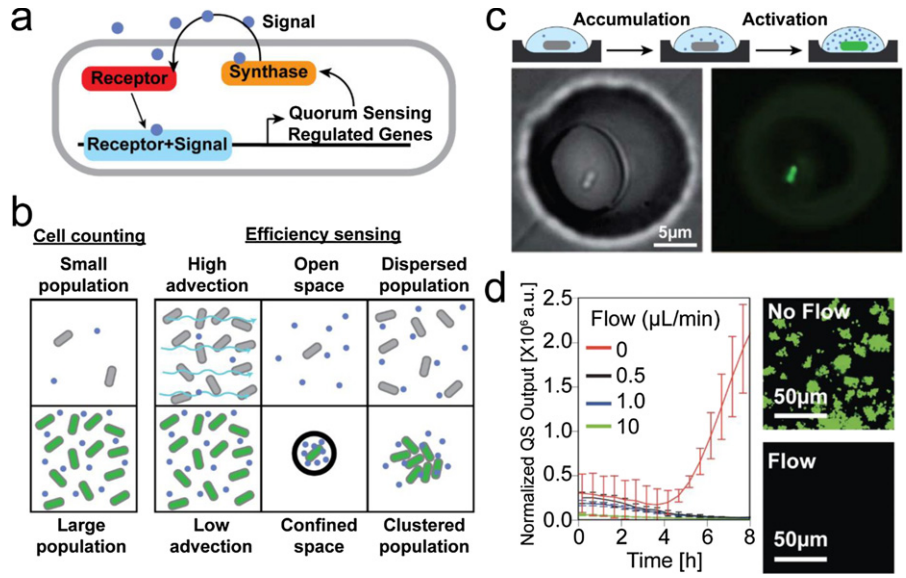

Figure 1. Bacteria use quorum sensing to gather information from the environment. (a) An illustration of a canonical quorum sensing scheme whereby a cell synthesizes a signal with a synthase, the signal diffuses into the extracellular environment, and signal re-enters the cell to bind to a receptor, which then regulates gene expression. (b) An illustration of quorum sensing as a means to count cells and to compute the efficiency of signal accumulation. In large populations, signals (blue dots) accumulate to a high concentration and cells respond by altering gene expression (green cells). Signal also accumulates to high concentrations in contexts of low advection, confinement, or clustered spatial distributions leading to expression of quorum sensing regulated genes. (c) An experiment to trap a single cell in a high cell density microdroplet, from Boedicker *et al* (2009) [21]. Optical (left) and fluorescent (right) microscopy images of a single cell expressing *gfp* after accumulation of released quorum sensing signals in the microdroplet. (d) Data from Kim *et al* (2016) [25], demonstrating that flow impedes quorum sensing. Fluorescent microscopy images showing cells with a quorum sensing-regulated fluorescent reporter grown with and without flow.

localized, potentially giving rise to complex and intricately structured communication networks. Years of experimental and theoretical studies provide insight into the ways molecular exchange enables cells to monitor and respond to the microscale spatial structure which they inhabit [31–33].

A simple example of cells responding to spatial structure is colony formation on solid media plates. When a small population of cells is spread onto a solid agar plate to grow into individual colonies, the number of cells and interactions between the cells influence the size and shape of the colonies that form. In this way, the larger scale patterns of growth are determined by the spatial distribution of cells on the plate. The patterns are shaped by depletion of nutrients as well as the exchange of signaling molecules, as in the case of *Paenibacillus dendritiformis* where the production of secondary metabolites by sister colonies biased the direction of colony growth [34]. Anyone that has spread cells onto an agar plate has observed an inverse relationship between the number and size of the colonies that grow. More colonies deplete the shared nutrient source inside the agar gel resulting in smaller colonies [35].

Spatial computations can also be programmed using synthetic biology. In one system, a mixture of positive and negative feedback on the production of diffusible signals self-regulated the relative size of the activated region of cells. For example, a cell was programmed to sense—in space and time- the amount of space available for growth and to scale the spatial pattern of activity accordingly [36]. The emergent bulls-eye pattern exhibited scale-free behavior between the width of the ring and the initial colony size, as shown in figure 2.

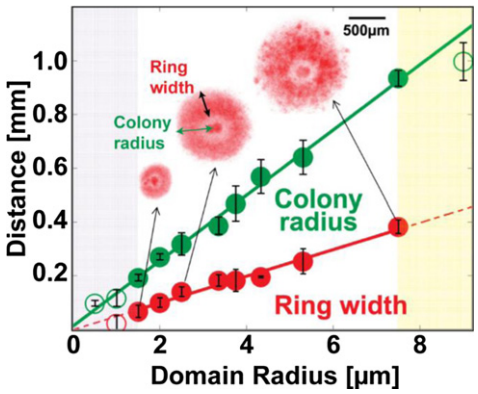

Figure 2. Signal exchange for spatial computations. Data and fluorescent microscope images (inset) from Cao *et al* (2016) [36], illustrating synthetically engineered colonies use signal exchange to calculate the spatial structure of the environment and form a scale invariant spatial pattern of gene expression. Distance refers to either colony radius (green data) or ring width (red data).

3. Computation of community composition

Signaling in realistic contexts is more than just production and detection of a single signaling

molecule. Not only do many bacterial species contain multiple signaling pathways, but multiple species or strains in the same environment use chemically similar signals for communication. Recent work has examined how bacteria within diverse populations are able to integrate information from mixtures of signals, and in the process, gain information about the composition of the local bacterial community.

3.1. Signal crosstalk and interference

The majority of bacterial signaling studies have focused on individual signaling pathways in isolation. It has been recognized for many years, however, that many signaling pathways can be influenced by the activity of neighboring cells [37]. In some cases neighboring cells produce enzymes that chemically modify or destroy released signaling molecules, thereby interfering with signal transduction [38]. Other species may detect molecular signals from their neighbors without producing any signals of their own [39]. It is also known that receptor proteins bind and respond to multiple chemical variants of a signal molecule, resulting in crosstalk between bacterial species producing distinct yet chemically similar signaling molecules

Crosstalk generally refers to the response of a cellular signaling network to a non-cognate signal from either the same species or strain or to signals from different species or strains. In the context of quorum sensing, *signaling crosstalk* refers to the binding of a receptor by a chemically similar but distinct signaling molecules that alters the downstream response of the cell. For example, when cocultured together, signal from *P. aeruginosa* induced expression of quorum sensing-regulated genes within *Burkholderia cepacia* [40]. Signaling crosstalk can excite or inhibit gene activation and does so to variable degree, dependent on the signal and receptor molecules. Such is the case in figure 3(a), where a receiver strain expresses quorum sensing-regulated genes sooner (excitatory response) or later (inhibitory response) depending on signal produced by a neighboring strain. Receptor proteins may exhibit promiscuous behavior, integrating a mélange of signaling molecules from various species. Early work on the promiscuity of quorum sensing receptors revealed that receptor proteins are capable of recognizing and responding to multiple signal variants [41]. Signal crosstalk has been reported in a variety of bacterial signaling systems, including acyl-homoserine lactones (AHL) and autoinducing peptides [42–44], and the strength of crosstalk has been quantified in detail for several systems [42, 45]. These studies reveal a rich diversity of excitatory and inhibitory crosstalk of varying strength, including neutral crosstalk in which a signal variant has no measurable influence on gene regulation. Crosstalk has not been accurately predicted from molecular structure and receptor sequence, but general trends have emerged such as the degree of chemical similarity tends to indicate stronger activating crosstalk [43].

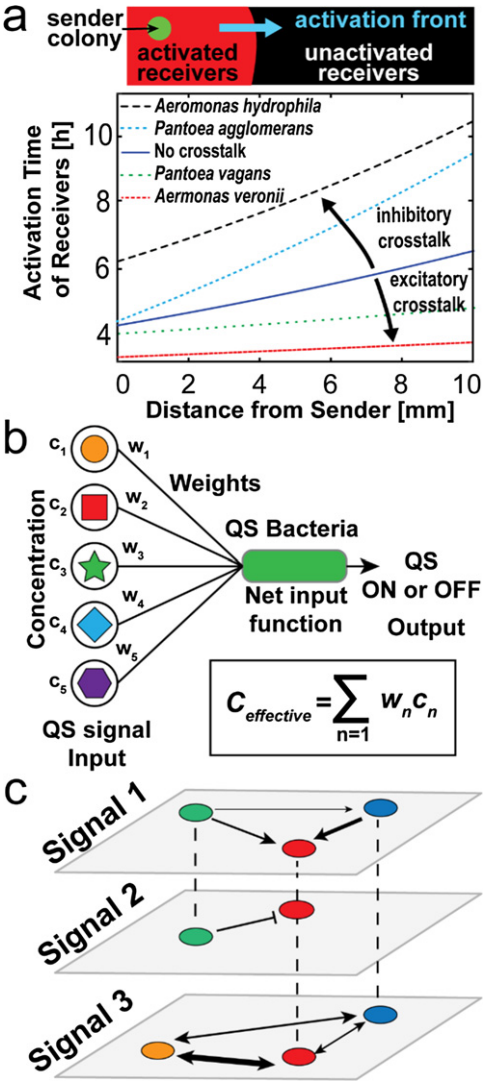

Figure 3. Quorum sensing to gather information about community composition. (a) (Top) An illustration of the sender–receiver experiment from Silva *et al* (2017) [42]. (Bottom) Measurements revealed neighboring strains inhibit or excite quorum sensing. (b) An illustration of quorum sensing as a neural net, whereby signals (inputs) of different concentrations with varying weights are integrated by a cell to compute a response of 'ON' or 'OFF' [51]. (c) A hypothetical illustration of a multilayer signaling network within a 4-species bacterial community. Each node represents a bacterial species, and each layer represents an orthogonal type of signal. Strains utilizing multiple signals appear on multiple layers. Arrows represent activation, flat headed arrows represent repression. Such models should aid in understanding the consequences of signal exchange within complex, microbial communities.

An open question remains about whether receptors have evolved specifically to take advantage of crosstalk. It has been proposed that crosstalk will naturally evolve due to the fitness cost of signaling and the presence of cheating within bacterial communities [46]. Crosstalk would provide additional information about the species or strains of cells that may be present. Theoretical work has shown that

inference of local community composition is a key benefit to populations which utilize quorum sensing [47]. Signaling pathways may have evolved to activate earlier or not at all in the presence of specific neighboring strains. For example, it has been suggested that crosstalk between *Streptococcus pneumoniae* might enable individual strains to anticipate production of antibiotic compounds by neighboring strains [48]. While a degenerate or non-cognate response to signal may appear sub-optimal, crosstalk can improve the ability of cells to sense their environment. Carballo-Pacheco *et al* demonstrated that crosstalk is a broadly optimal strategy for signaling [49]. Crosstalk might also enable some bacteria to cooperate, by activating specific phenotypes only in the presence of combinations of signals from neighboring cells. For example, crosstalk between three species found on olive trees was found to increase the ability of one of these species, *Pseudomonas savastanoi*, to cause an infection [50]. More work is needed to fully elucidate the potential benefits of crosstalk within diverse bacterial communities.

3.2. Microbes as a neural network

Looking beyond signal exchange between two species or strains, recent work more formally analyzed how signal exchange within diverse cellular communities influences collective decision making [51]. Distributed, diverse bacterial communities adaptively integrating and processing information constitute a neural network. Neural networks were originally inspired by networks of interconnected neurons [52], whereby nodes are connected to each other to reflect their mutual influence. The population of each species or strain of bacteria is a node, the connections between nodes represent signal exchange, and the weight of each connection represents the strength and type of signal crosstalk. Each cell within the network measures the mixed concentration of external signals, thereby probing the activity state and density of each cell type in the network, as depicted in figure 3(b). The model can be used to predict the quorum sensing activity of multiple bacterial strains within a mixed community of cells.

The utility of this neural network model to predict the community-level signaling state of a bacterial network was shown in a recent publication by Silva and Boedicker [51]. In this study, communication was analyzed in a naturally occurring community of *Bacillus subtilis* strains, each producing a different variant of the ComX quorum sensing signal [53]. The pairwise crosstalk terms were measured for each combination of species, enabling full reconstruction and prediction of activity within the 5-strain network. Quorum sensing activity of each strain was accurately predicted for different combinations of all five signals, revealing that even small changes in one of the signals could modulate the community-level signaling state. These results demonstrate how the community of strains uses a combination of multiple signals to compute an activity state for each member of the community, thus setting an activity pattern for the community. Further theoretical developments applying the neural network model to bacterial quorum sensing revealed how the number of attractor states, the community-level profile of quorum sensing activity, depended on the strength of interactions and the community composition [54].

Through the same approach, response to multiple types of signals in a single species also could be analyzed. For example, in *P. aeruginosa* intracellular levels of c-di-GMP are altered in response to extracellular stimuli such as chemoattractants, which could be a signal for motility, or mechanical contact with surfaces, which could be a signal for biofilm formation. As a response to the levels of c-di-GMP, genes controlling flagella needed for swarming motility and extracellular matrix genes needed for biofilm formation are either repressed or activated. Yan *et al* [55], modeled the binary response to the intracellular secondary messenger c-di-GMP in *P. aeruginosa as a bow-tie network*. The network integrates each signal with a different weight and responds to the net signal through a nonlinear function. Through this perspective, the architecture of c-di-GMP works as a machine learning classifier whose function is to determine, from a set of stimuli, to which of two categories an environment belongs—biofilm—favoring or motility-favoring. The fittest network is the one with the highest geometric mean of fitness across multiple environments, equivalent to a logistic regression criterion. A result of such analysis, knowledge of the number of sensors in the network enables prediction of the evolutionary history of *P. aeruginosa*.

The theoretical approach of neural networks may be widely applicable to the study of natural quorum sensing communities. Natural bacterial ecosystems are extremely diverse, containing thousands of species and strains of bacteria capable of diffusive signal exchange. Estimates made using metagenomics approaches found that 8% of cells in one soil ecosystem were capable of participating in the exchange of AHL signals [56]. In another study, the potential to participate in AHL signaling was found in 40% of the 129 bacterial species isolated from the cottonwood tree [57]. It would appear typical that individual cells receive signaling input from several neighboring species. Examples of crosstalk between multiple strains has been reported for both *S. pneumoniae* and *S. aureus* [58, 59]. Given that in many contexts quorum sensing is associated with outcomes such as virulence or biofilm formation, understanding community-level influences on activity patterns and the potential benefits of such regulatory schemes will be an important direction for future research.

3.3. Integration of stimuli in multilayer networks

Mapping the exchange of several chemically related signaling molecules to a network of interactions between multiple species or strains of bacteria will facilitate the prediction of output states of the community. Real systems, however, include interdependencies that are not easily understood as a single layer network: many species or strains may participate in multiple signaling networks simultaneously. To account for this complexity, a more general framework, in which different networks evolve or interact with each other, is needed. These are known as multilayer networks, in which each layer encodes a specific type of information about the system [60]. Adding an additional degree of freedom to the system, namely layers, might reveal the information encoded in the network that cannot be captured otherwise.

From this view, one might be able to map communication in a diverse bacterial community to a multilayer network, as depicted in figure 3(c). In such a network, each layer represents a class of signaling molecules. Nodes on each layer represent bacterial species or strains that directly respond to or produce the type of signal specified for that layer linked through weighted, directed edges. Layers of such a network might represent orthogonal types of chemical signals that cells use to communicate. It is known that some bacteria, such as *Vibrio harveyi*, utilize multiple types of chemical signals and integrate those input signals into a common pathway [61]. Therefore some bacterial species will appear as connected nodes on multiple layers of the network. Although signal crosstalk within microbial communities has not yet been analyzed using a multilayer network framework, work on biological communication in eukaryotic cells, including neurons, reveals the utility of this approach.

The multilayer network formalism more clearly captures the myriad of connections present within complex interaction networks. A multilayer networks framework has been applied to study tissue development in *Caenorhabditis elegans,* cancer complexomes, and protein-protein interaction networks [62–64]. Not surprisingly, complex interaction networks such as the human brain have been modeled as a multilayer network. De Domenico *et al* [65, 66] analyzed the connectivity among different regions of the human brain through multilayer network analysis. Different brain regions are nodes connected through the exchange of signals, represented as edges. Layers correspond to different frequency intervals corresponding to these signals. The results of their study revealed that hubs in multilayer networks are, in general, different from the hubs identified by standard methods based on single-layer network analysis. Such hubs enabled distinction between the brain of a schizophrenic patient from a healthy brain in resting state. Could similar clusters of species within a microbial ecosystem play an important role in setting the activity of large groups of bacterial species? For bacterial signaling networks, a multilayer analytical approach should elucidate how coupled signal transduction within individual species impacts the community-wide response to signal exchange and perturbations. Analysis of bacterial networks may identify new examples of emergent phenomena and criticality, thought to be ubiquitous among living networks [67].

4. Optimization of signaling

The computations performed by bacterial signal exchange have an energetic cost, suggesting that evolution has likely increased the efficiency of signal exchange. Efficient signal exchange involves reduction in the cost of signaling components, and also incorporates aspects of optimizing information transfer. How is quorum sensing an efficient mechanism of information transfer? Recent work has begun to analyze bacterial communication from the perspective of information transfer and energetic efficiency.

4.1. Energetic costs of communication

Gathering meaningful information from the environment carries a significant expense. Broadly, storing information in a biomolecule or macromolecule demands a change in free energy in the system, which may be gathered from the environment through metabolism. Berg and Purcell [68], famously demonstrated that the information gathered by a cell is proportional to the cell's uncertainty in the external concentration and limited by the stochastic flux in the occupancy of the outer membrane receptors. Mehta and Schwab [69], build on that work by examining the tradeoff between energy spent by the cell and the information gathered by a two-component system. Their work revealed a limit on chemosensing, namely, the metabolic budget of the cell, as shown in figure 4(a). As the uncertainty in the measurement of the external signal concentration decreases the power consumption likewise increases. The important finding overall is that learning requires energy, which places a strong constraint on biochemical networks in bacteria.

A similar cost for bacterial signal exchange has also been measured. Ruparell *et al* [70], examined the metabolic strain of autoinducer synthesis by comparing growth rates of wild-type *E. coli* and two strains expressing LasI and RhlI, quorum sensing signal synthases native to *P. aeruginosa*. The strains expressing LasI and RhlI grew slower than the strain that did not, as shown in figure 4(b). This study revealed that participation in the production of quorum sensing signals had the cost of a reduced growth rate.

Given that the computations performed by bacterial signal exchange are both energetically costly and typically involve large populations of cells

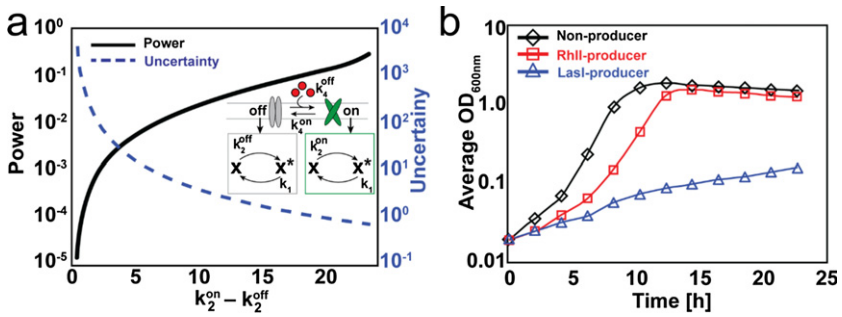

Figure 4. Energetic aspects of gathering information. (a) Theoretical results from Mehta and Schwab (2012) [69] representing the idea that cells use energy to reduce the uncertainty in information collected from their environment, in this case using a two-component system (inset). (b) Experimental data from Ruparell *et al* (2016) [70], showing that cells that express the RhlI and LasI enzymes, which produce autoinducer signal, have reduced growth rates.

working in a coordinated fashion, it is likely that cheaters would emerge. Cheaters are cells that do not contribute to the production of public goods but still benefit from the work of others, thus gaining a fitness advantage over the cooperators. In the context of bacterial computations, the information gained from signal exchange can be viewed as a public good, and cheater cells potentially monitor signal concentrations without the energetic investment into the full information gathering process. In a theoretical study Schuster *et al* [71], showed cooperation is evolutionarily selected for when the metabolic burden of a public good is low. At intermediate metabolic costs, cooperators and cheaters coexist. At high cost, all cells abandon cooperation. Given that information gathering via quorum sensing is viewed as having a relatively low cost, that would suggest quorum sensing cheaters are tolerated in most contexts. It is interesting to note that the majority of quorum sensing components identified in genomes contain only a receptor protein, an orphan receptor, without an associated synthase protein [72]. Cheating in the context of public goods is a well-established field [73–75], and future work should consider quorum sensing signals in the environment, from which cells can learn about local physical and biological conditions, as a form of public good.

4.2. Measuring information flow

Information theory framework enables us to better address questions related to the efficiency of signal exchange. From this perspective, signaling process is assumed to be a black box with external concentrations as input and phenotypic response as the output of the system. Mutual information is commonly used to quantify how much information cells can extract from an external stimulus [76–78]. Calculating mutual information often requires a precise knowledge of the input signal distribution which, for individual bacteria, is typically not known. To resolve this issue with mutual information, channel capacity is used instead. Channel capacity is defined as the maximum possible information that a communication channel can carry, the supremum of mutual information over all possible choices of the input probability distributions [79]. Both mutual information and channel capacity are generally calculated in bits, which gives a sense for how many yes or no decisions can be made by the cell.

Theoretical work has examined information exchange within biological systems. Hong *et al* [105], modeled the response in a generic signaling system as a sigmoid function of signal concentrations, with a Gaussian noise term centered at zero to account for intrinsic noise. Such simplified dose–response analysis is ubiquitous in many real signaling motifs. Having intrinsic noise dependent on protein copy numbers in such system, the authors estimated the channel capacity for a variety of transmembrane proteins, with copy numbers per cell ranging from 10^2 to 10^5. Their results showed signaling motifs prone to intrinsic noise can transmit of 4–6 bits of information. Experimental measurements of the signaling systems studied to date encode less than 2.5 bits of information, with the majority transmitting significantly less than 1 bit (capacity of a binary switch). As a result, extrinsic noise plays the major role in information integration.

Although noise presumably diminishes the information transmission in signaling systems, it has been shown that noise affects information transmission in a more convoluted way. Rodrigo and Poyatos [80], modeled the output of a simple signaling motif as ordinary differential equations with addition of Gaussian noises terms accounting for intrinsic and extrinsic noises. The authors showed mutual information between the distribution of such noisy output and a given uniform distribution of inputs does not always decrease with noise, however mutual information always decreases with respect to the amplitude of extrinsic noise. Certain amplitudes of intrinsic noise can even amplify the information transmission.

A few experimental efforts to date have examined the information capacity of bacterial communication [45, 81, 82]. Mehta *et al* [81], applied an

information theoretic approach to resolve why *V. harveyi* possess two similar quorum sensing channels responsible the same downstream regulator. The channels differ in their use of input, AI-1, specific to *V. harveyi*, and AI-2, shared among many bacterial species. Because the cells respond almost equivalently to both signals, the channel, at first glance, encoded 0.8 bits, which is not sufficient to detect two environmental states. Upon further inspection, however, *V. harveyi* increases the channel capacity to ∼1.5 bits by producing AI-1 and AI-2 at the same rate, which ensures that the extracellular concentration of AI-2 is greater than or equal to the concentration of AI-1, which only *V. harveyi* produces. Moreover, *V. harveyi* could increase the channel capacity to ∼1.5 bits by positively regulating the number of AI-2 receptors. This strategy allows *V. harveyi* to preferentially learn about AI-2 at low cell density and about AI-1 at high cell density.

Pérez *et al* [45] studied noise and crosstalk *Vibrio fischeri*, which produces two quorum sensing signals. Their experimental measurements along with mutual information analysis showed the mutual information between the signal inputs and the lux output is less than one bit. Furthermore, they showed the lux genes in *V. fischeri* do not appear to distinguish between the two HSL inputs, and even with two signal inputs the regulation of *lux* is extremely noisy. Hence the role of crosstalk from the C8-HSL input may not improve sensing precision, but rather suppresses the sensitivity of the switch for as long as possible during colony growth.

A recent paper examined how reducing noise in the output signal might improve information transmission [102]. The study focused on the transcription factor LacI, which responds to changes in lactose availability. The authors implemented a minimal model for the information processed by a repressor gene circuit. They related channel capacity, repressor copy number, and repressor-DNA binding affinity, as shown in figure 5(a). A circuit with zero repressors is a circuit with zero channel capacity, since the gene is constitutively expressed. As the number of repressors increases the channel capacity increases. At a very high repressor number, however, the channel capacity decreases since the gene would be indefinitely repressed. A complementary interpretation is that zero-channel capacity is a consequence of the circuit having an overlapping input–output function whereas more separated input and output distributions imbue higher channel capacity to the circuit. The change in channel capacity and number of bits encoded strongly depends on the binding affinity of the repressor to the DNA. Notably they find that as the repressor concentration increases the cell to cell variation in the number of repressors, the intrinsic noise, decreases. There remains more to discover regarding the ability of biological systems to exchange information, and continued dialogue between information theory and quantitative single-cell measurements should prove fruitful in the coming years.

4.3. Single-cell heterogeneity in communication

Heterogeneity in the activity of individual cells impacts the efficiency of communication within cellular populations. Single-cell variability can be the result of both genotypic variation and phenotypic variation of genetically identical cells [83]. Heterogeneity has been reported in bacterial communication systems. In the studies of quorum sensing of confined cells, significant variability in the expression of quorum sensing-regulated genes was observed in *P. aeruginosa* cells [21]. Only 20% of cells upregulated quorum sensing-controlled genes within small populations, although the molecular mechanism causing such variability was not reported. Measurements of heterogeneity in the expression of quorum sensing related genes have been shown for *V. harveyi* [84]. *V. harveyi* strains lacking the genes for signal production were engineered to contain a transcriptional fusion of *gfp* fused to a quorum sensing responsive reporter. Upon addition of exogenously added signal, the response of individuals within the population varied, as shown in figure 5(b). Variability in quorum sensing activation has been shown to benefit group behaviors, such as biofilm formation [85]. Similar variability was observed in *V. fischeri* and *Listeria monocytogenes* [86, 87]. Heterogeneity in expression of quorum sensing-regulated genes was also examined in genetically identical population of *Sinorhizobium fredii*. Here the amount of heterogeneity in expression levels depended on the gene analyzed, and the extent of variability in single-cell expression levels were modulated by environmental factors [88]. In wild quorum sensing populations, genetic variation has been reported, with about 20% of *P. aeruginosa* isolates containing variability in the genomic sequence of the *lasR* receptor protein [89]. It remains unclear exactly how information transmission within bacterial populations is affected by heterogeneity at the single-cell level.

A relatively unexplored aspect of single-cell variability is the coupling of phenotypic variability within multi-species populations. Are there important consequences for populations when two rare phenotypes of different species interact? One recent experiment to report on this concept [90], examined the coupling of growth rates within small, mixed populations of *E. coli* and *Enterobacter cloacae*. Using a microwell device to create replicate groups containing only a few cells of each species, it was found that the mean and variability of growth rates was higher in co-cultures than single strain cultures. More work is needed to understand how single-cell variability, specifically heterogeneity in communication pathways, changes in diverse environments composed of multiple species. These effects would be enhanced

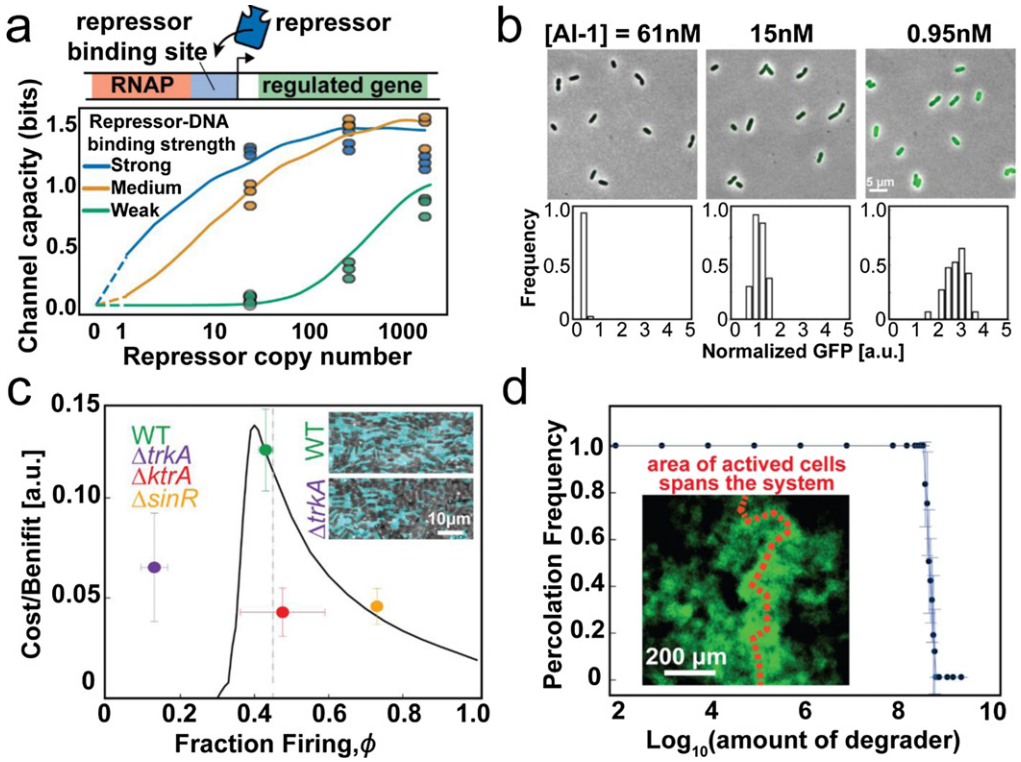

Figure 5. The efficiency of collective behaviors in bacteria. (a) Information content of a gene circuit in *E. coli*, as reported by Razo-Mejia and Phillips (2019) [92]. Theoretical predictions closely matched experimental measurements of the channel capacity as a function of the number and binding strength of the repressor protein. Given the energetic costs of gathering information, cells may evolve to maximize information theoretic metrics such as channel capacity. (b) (Top) Overlaid phase and fluorescence microscopy images of *V. harvyei* from Long *et al* [84]. The cells were engineered to express *gfp* in response to exogenously added quorum sensing signal AI-1, which represses gene expression. The concentration is stated above each image. (Bottom) Normalized histograms depicting the fluorescence per cell highlighting heterogeneity of *gfp* expression among genetically identical cells. (c) Overlaid fluorescent microscope images (inset) from Larkin *et al* [93], revealed heterogeneity in the opening of potassium channels within *B. subtilis* biofilms. Cells achieved long-range communication even when only a fraction of the cells participated in potassium ion exchange. Wild-type cells operated near a percolation transition, which maximized the cost to benefit ratio of communication. (d) Data and fluorescent microscope image (inset) from Silva *et al* (2019) [94], demonstrating a percolation transition in the expression of quorum sensing-regulated genes. Beyond a critical number of cells that degraded the signal, connected regions of activated cells no longer spanned the network.

in fragmented populations in which individual cells are not sampling population averages, but instead interact locally with neighbors sampling their own phenotypic distributions [91].

4.4. Spatial self-organization and percolation improve the efficiency of cellular communication

Given the strong influence of spatial structure on the activity of cellular populations, it is not surprising that many populations have been shown to self-regulate spatial structure to optimize molecular exchange. Such modulation of spatial structure impacts the efficiency of information flow within bacterial populations.

Most examples of emergent spatial structures are in the context of nutrient acquisition. A population of *B. subtilis* utilizing an extracellular public good relied on mobility of the cells to self-organize into a spatial pattern that optimized growth [95]. The impact of the public good, an enzyme that processed a complex food source, was highly non-linear, requiring a high density of cells before enzyme production influenced the growth rate. When seeded at low density, cells were driven to motile states and self-organized into high density colonies in order to survive. The same study examined the relationship of spatial patterning and cooperation by growing cells in an environment with glucose, a resource that could be used without community wide action. In these conditions, no distinct colonies form. Decreasing the concentration of glucose, however, drove the cells toward distinct high-density colonies.

In other examples, spatial structures optimized the diffusive exchange of metabolites within mixed microbial populations [96, 97]. A coculture of *Pseudomonas putida* and *Pseudomonas veronii* was found to spatially segregate and organize as a result of food and oxygen gradients [98]. The spatial organization of marine bacteria was essential for degradation of external food particles [99]. Cocultures of engineered yeast strains also exhibited self-generated spatial structure related to metabolic interactions between strains [100]. A pair of bacterial species with linked metabolic pathways have even been found to have coevolved mechanisms to adopt specific spatial structures when cocultured on surfaces [101].

These are a few of the many reported examples of the intimate connection between metabolite exchange and spatial structure. Signaling efficiency is also strongly dependent on spatial structure. Darch *et al* studied quorum in aggregates of *P. aeruginosa* cells which were confined and spatially positioned using a microscale 3D-printing platform. Aggregates containing 2000 signal-producing cells were unable to signal neighboring aggregates, while those containing $\geqslant$5000 cells communicated with neighbors as far away as 176 μm. These findings highlighted the dependence of efficient communication on spatial structure [32]. Work on *V. harveyi* cells loaded into hydrogel microcapsules of various sizes reached a similar conclusion. Large aggregates of cells, with a size of approximately 25 μm, accumulated many more autoinducers than did small aggregates with a size of approximately 10 μm, thus demonstrating that the process of quorum sensing relies on the spatial structure of the population [31]. These studies manipulated spatial structure using laboratory methods, but there are likely similar examples of spatial self-organization to optimize signaling and group coordination within bacterial ecosystems.

Recently the concept of percolating networks has been discussed in the context of cellular communication. Cells operating near the threshold of a percolation transition can improve the efficiency of long-distance communication within a population of cells. In a percolating network, nodes or cells are distributed on a spatial grid. Each cell has an activity level, and the percentage of activated cells on the grid strongly influences the spatial range and overall activity level of the entire network. When only a few cells are activated, activated cells are isolated and do not form large patches. Above a critical percentage of activated cells, the activated cells form an interconnected network that spans a very large range, often the entire length of the network. This transition in the connectivity of activated regions has been reported in several biological contexts, including embryo development [102]. Percolation networks often operate near a critical threshold or phase transition, below which only short-range clusters interact and above which long-range or system-spanning conduits emerge. Here we will focus on two examples of percolating networks involving bacterial signal exchange.

Larkin *et al* explored such behavior in biofilms of *B. subtilis* [93], where individual cells open and close ion channels to communicate. Starved cells in the interior of the biofilm send K^+ to neighbors, stalling their metabolism, until the signal wave meets the edge of the biofilm where resources are available. The reduced consumption along the ion wave provides more nutrients for the stressed, interior cells, increasing the fitness of the entire population. A minimal threshold percentage of cells participating in K^+ signaling led to the formation of system spanning channels. A wild type *B. subtilis* biofilm operated near criticality, balancing the linear cost of signaling with the highly non-linear benefit received, see figure 5(c).

Quorum sensing similarly can exhibit a percolation transition. Spatial patterns of the expression of quorum sensing-regulated gene expression were studied in a synthetic two-strain community composed of a signal producing strain and a signal degrading strain [94]. The signal producing strain released C4-AHL and responded to a high concentration of signal by producing a fluorescent reporter protein. The signal degrading strain produced an enzyme that degraded the signal, thus acting as a sink for the signal. When mixed together, the size and connectivity of the activated regions producing GFP depended on the ratio of two strains. Above a critical ratio, the activated cells formed a connected region that spanned the entire system, as shown in figure 5(d). The activated regions also followed scaling laws expected for such percolating networks [103], demonstrating that the size and distribution of active regions within a spatially dispersed population could be predicted from fundamental physical concepts. Long-range coordination of signaling states was also demonstrated, even in the presence of interference from a neighboring strain.

5. Future perspectives

Recent insights into the ability of bacterial communities to gather information from the environment and coordinate large-scale behavior should enable the development of strategies to both control diverse populations of microbes and design multispecies communities for new applications in biotechnology. In recent years the advantages of division of labor within both synthetic and natural microbial consortia have been reported [104–108]. The viability of these approaches requires that communities are able to maintain relatively stable community composition over time. Several strategies have been proposed to balance interactions within multispecies communities, including designed cross feeding of metabolites and the use of spatial niches to maintain diversity [109, 110]. What is often missing in this focus on metabolic balance, is whether or not cells will maintain cellular activities of interest, other than growth of course, due to often poorly defined regulatory interactions between community members. As highlighted here, signaling interactions between species are likely commonplace in natural biological contexts. Much more work is needed to reveal how populations of cells learn about their surroundings to modify their behaviors, and how such regulation within a community context is beneficial to both individual species or strains and the community as a whole. Theoretical approaches described above should help advance our

understanding and prediction of how communities of cells communicate and regulate activity within biologically diverse contexts. Analysis of how populations of cells gather information and regulate activity, bacterial computations, will help identify strategies that maintain functional characteristics as well as species composition within communities. A deeper understanding of how regulatory interactions are influenced by both spatial structure and single-cell heterogeneity will be essential in many contexts and may lead to new strategies for control and stability. Next steps should include engineered communities capable of self-regulating and adapting to changes in biological, chemical, and physical conditions. These approaches should incorporate cells using signal exchange to gather information about local physical and biological conditions to calculate an appropriate response, a process that has already evolved in many natural communities to maintain both diversity and function despite significant uncertainty in conditions.

A lofty goal, which hopefully will become more realistic from advances in the design of engineered communities, is the prediction and control of community function in the wild. Given the tremendous diversity of real microbial ecosystems, it would appear that an exhaustive mapping of species interactions, whether metabolic or regulatory, is impractical. An ideal solution to this problem would be the identification of the general rules for how cells gather information to set activity levels for each species and strain [82, 111], regardless of the specific community composition or the activity of interest. Future work focused on universal strategies and limitations of bacterial community computation should help elucidate such rules.

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References

[1] Ross-Gillespie A and Kümmerli R 2014 Collective decision-making in microbes *Front. Microbiol.* **5** 54
[2] Ulrich L E, Koonin E V and Zhulin I B 2005 One-component systems dominate signal transduction in prokaryotes *Trends Microbiol.* **13** 52–6
[3] Mitrophanov A Y and Groisman E A 2008 Signal integration in bacterial two-component regulatory systems *Genes Dev.* **22** 2601–11
[4] Galperin M Y 2005 A census of membrane-bound and intracellular signal transduction proteins in bacteria: bacterial IQ, extroverts and introverts *BMC Microbiol.* **5** 35
[5] Waters C M and Bassler B L 2005 Quorum sensing: cell-to-cell communication in bacteria *Annu. Rev. Cell Dev. Biol.* **21** 319–46
[6] Boedicker J and Nealson K 2015 Microbial communication via quorum sensing *IEEE Trans. Mol. Biol. Multi-Scale Commun.* **1** 310–20
[7] Miller M B and Bassler B L 2001 Quorum sensing in bacteria *Annu. Rev. Microbiol.* **55** 165–99
[8] Hastings J W and Greenberg E P 1999 Quorum sensing: the explanation of a curious phenomenon reveals a common characteristic of bacteria *J. Bacteriol.* **181** 2667–8
[9] Schuster M, Joseph Sexton D, Diggle S P and Peter Greenberg E 2013 Acyl-homoserine lactone quorum sensing: from evolution to application *Annu. Rev. Microbiol.* **67** 43–63
[10] West S A, Winzer K, Gardner A and Diggle S P 2012 Quorum sensing and the confusion about diffusion *Trends Microbiol.* **20** 586–94
[11] Nealson K H, Platt T and Hastings J W 1970 Cellular control of the synthesis and activity of the bacterial luminescent system *J. Bacteriol.* **104** 313
[12] Weber M and Buceta J 2013 Dynamics of the quorum sensing switch: stochastic and non-stationary effects *BMC Syst. Biol.* **7** 6
[13] Williams P and Cámara M 2009 Quorum sensing and environmental adaptation in Pseudomonas aeruginosa: a tale of regulatory networks and multifunctional signal molecules *Curr. Opin. Microbiol.* **12** 182–91
[14] Ng W-L and Bassler B L 2009 Bacterial quorum-sensing network architectures *Annu. Rev. Genet.* **43** 197–222
[15] Popat R, Cornforth D M, McNally L and Brown S P 2015 Collective sensing and collective responses in quorum-sensing bacteria *J. R. Soc. Interface* **12** 20140882
[16] Moreno-Gámez S, Sorg R A, Domenech A, Kjos M, Weissing F J, van Doorn G S *et al* 2017 Quorum sensing integrates environmental cues, cell density and cell history to control bacterial competence *Nat. Commun.* **8** 854
[17] Bai A J and Rai Vittal R 2014 Quorum sensing regulation and inhibition of exoenzyme production and biofilm formation in the food spoilage bacteria *Pseudomonas psychrophila* PSPF19 *Food Biotechnol.* **28** 293–308
[18] Barnard A M L, Bowden S D, Burr T, Coulthurst S J, Monson R E and Salmond G P C 2007 Quorum sensing, virulence and secondary metabolite production in plant soft-rotting bacteria *Phil. Trans. R. Soc.* B **362** 1165–83
[19] Rajput A, Kaur K and Kumar M 2015 Sigmol: repertoire of quorum sensing signaling molecules in prokaryotes *Nucleic Acids Res.* **44** D634–9
[20] Hense B A, Kuttler C, Müller J, Rothballer M, Hartmann A and Kreft J-U 2007 Does efficiency sensing unify diffusion and quorum sensing? *Nat. Rev. Microbiol.* **5** 230–9
[21] Boedicker J Q, Vincent M E and Ismagilov R F 2009 Microfluidic confinement of single cells of bacteria in small volumes initiates high-density behavior of quorum sensing and growth and reveals its variability *Angew. Chem., Int. Ed.* **48** 5908–11
[22] Carnes E C, Lopez D M, Donegan N P, Cheung A, Gresham H, Timmins G S and Brinker C J 2010 Confinement-induced quorum sensing of individual Staphylococcus aureus bacteria *Nat. Chem. Biol.* **6** 41–5
[23] Qazi S N A, Counil E, Morrissey J, Rees C E D, Cockayne A, Winzer K, Chan W C, Williams P and Hill P J 2001 Agr expression precedes escape of internalized Staphylococcus aureus from the host endosome *Infect. Immun.* **69** 7074–82
[24] Emge P, Moeller J, Jang H, Rusconi R, Yawata Y, Stocker R *et al* 2016 Resilience of bacterial quorum sensing against fluid flow *Sci. Rep.* **6** 33115

[25] Kim M K, Ingremeau F, Zhao A, Bassler B L and Stone H A 2016 Local and global consequences of flow on bacterial quorum sensing *Nat. Microbiol.* **1** 15005

[26] Kirisits M J, Margolis J J, Purevdorj-Gage B L, Vaughan B, Chopp D L, Stoodley P and Parsek M R 2007 Influence of the hydrodynamic environment on quorum sensing in Pseudomonas aeruginosa biofilms *J. Bacteriol.* **189** 8357–60

[27] Cornforth D M, Popat R, McNally L, Gurney J, Scott-Phillips T C, Ivens A, Diggle S P and Brown S P 2014 Combinatorial quorum sensing allows bacteria to resolve their social and physical environment *Proc. Natl Acad. Sci.* **111** 4280

[28] Shapiro J A 2007 Bacteria are small but not stupid: cognition, natural genetic engineering and socio-bacteriology *Stud. Hist. Philos. Sci.* C **38** 807–19

[29] Dworkin M 2006 Prokaryotic life cycles *The Prokaryotes: Ecophysiology and Biochemistry* vol 2 ed M Dworkin, S Falkow, E Rosenberg, K-H Schleifer and E Stackebrandt (Berlin: Springer) pp 140–66

[30] Pompano R R, Chiang A H, Kastrup C J and Ismagilov R F 2017 Conceptual and experimental tools to understand spatial effects and transport phenomena in nonlinear biochemical networks illustrated with patchy switching *Annu. Rev. Biochem.* **86** 333–56

[31] Gao M, Zheng H, Ren Y, Lou R, Wu F, Yu W *et al* 2016 A crucial role for spatial distribution in bacterial quorum sensing *Sci. Rep.* **6** 34695

[32] Darch S E, Simoska O, Fitzpatrick M, Barraza J P, Stevenson K J, Bonnecaze R T, Shear J B and Whiteley M 2018 Spatial determinants of quorum signaling in a Pseudomonas aeruginosa infection model *Proc. Natl Acad. Sci. USA* **115** 4779–84

[33] Yusufaly T I and Boedicker J Q 2016 Spatial dispersal of bacterial colonies induces a dynamical transition from local to global quorum sensing *Phys. Rev.* E **94** 062410

[34] Be'er A, Zhang H P, Florin E-L, Payne S M, Ben-Jacob E and Swinney H L 2009 Deadly competition between sibling bacterial colonies *Proc. Natl Acad. Sci.* **106** 428–33

[35] Chacón J M, Möbius W and Harcombe W R 2018 The spatial and metabolic basis of colony size variation *ISME J.* **12** 669–80

[36] Cao Y, Ryser M D, Payne S, Li B, Rao C V and You L 2016 Collective space-sensing coordinates pattern scaling in engineered bacteria *Cell* **165** 620–30

[37] Rampioni G, Leoni L and Williams P 2014 The art of antibacterial warfare: deception through interference with quorum sensing-mediated communication *Bioorg. Chem.* **55** 60–8

[38] Dong Y-H, Xu J-L, Li X-Z and Zhang L-H 2000 AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora *Proc. Natl Acad. Sci.* **97** 3526–31

[39] Michael B, Smith J N, Swift S, Heffron F and Ahmer B M M 2001 SdiA of Salmonella enterica is a LuxR homolog that detects mixed microbial communities *J. Bacteriol.* **183** 5733–42

[40] Riedel K *et al* 2001 N-acylhomoserine-lactone-mediated communication between Pseudomonas aeruginosa and Burkholderia cepacia in mixed biofilms *Microbiol.* **147** 3249–62

[41] McClean K H *et al* 1997 Quorum sensing and Chromobacterium violaceum: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones *Microbiol.* **143** 3703–11

[42] Silva K P T, Chellamuthu P and Boedicker J Q 2017 Quantifying the strength of quorum sensing crosstalk within microbial communities *PLoS Comput. Biol.* **13** e1005809

[43] Wellington S and Greenberg E P 2019 Quorum sensing signal selectivity and the potential for interspecies cross talk *mBio* **10** e00146 e00219

[44] Wu F, Menn D J and Wang X 2014 Quorum-sensing crosstalk-driven synthetic circuits: from unimodality to trimodality *Chem. Biol.* **21** 1629–38

[45] Pérez P D, Weiss J T and Hagen S J 2011 Noise and crosstalk in two quorum-sensing inputs of Vibrio fischeri *BMC Syst. Biol.* **5** 153

[46] Eldar A 2011 Social conflict drives the evolutionary divergence of quorum sensing *Proc. Natl Acad. Sci.* **108** 13635

[47] Schluter J, Schoech A P, Foster K R and Mitri S 2016 The evolution of quorum sensing as a mechanism to infer kinship *PLoS Comput. Biol.* **12** e1004848

[48] Miller E L, Kjos M, Abrudan M I, Roberts I S, Veening J-W and Rozen D E 2018 Eavesdropping and crosstalk between secreted quorum sensing peptide signals that regulate bacteriocin production in Streptococcus pneumoniae *ISME J.* **12** 2363–75

[49] Carballo-Pacheco M, Desponds J, Gavrilchenko T, Mayer A, Prizak R, Reddy G *et al* 2019 Receptor crosstalk improves concentration sensing of multiple ligands *Phys. Rev.* E **99** 022423

[50] Hosni T *et al* 2011 Sharing of quorum-sensing signals and role of interspecies communities in a bacterial plant disease *ISME J.* **5** 1857–70

[51] Silva K P T and Boedicker J Q 2019 A neural network model predicts community-level signaling states in a diverse microbial community *PLoS Comput. Biol.* **15** e1007166

[52] Hopfield J J 1982 Neural networks and physical systems with emergent collective computational abilities *Proc. Natl Acad. Sci.* **79** 2554

[53] Ansaldi M, Marolt D, Stebe T, Mandic-Mulec I and Dubnau D 2002 Specific activation of the Bacillus quorum-sensing systems by isoprenylated pheromone variants *Mol. Microbiol.* **44** 1561–73

[54] Yusufaly T I and Boedicker J Q 2017 Mapping quorum sensing onto neural networks to understand collective decision making in heterogeneous microbial communities *Phys. Biol.* **14** 046002

[55] Yan J, Deforet M, Boyle K E, Rahman R, Liang R, Okegbe C, Dietrich L E P, Qiu W and Xavier J B 2017 Bow-tie signaling in c-di-GMP: machine learning in a simple biochemical network *PLoS Comput. Biol.* **13** e1005677

[56] Williamson L L, Borlee B R, Schloss P D, Guan C, Allen H K and Handelsman J 2005 Intracellular screen to identify metagenomic clones that induce or inhibit a quorum-sensing biosensor *Appl. Environ. Microbiol.* **71** 6335–44

[57] Schaefer A L, Lappala C R, Morlen R P, Pelletier D A, Lu T-Y S, Lankford P K, Harwood C S and Greenberg E P 2013 LuxR- and luxI-type quorum-sensing circuits are prevalent in members of the Populus deltoides microbiome *Appl. Environ. Microbiol.* **79** 5745–52

[58] Pinchas M D, LaCross N C and Dawid S 2015 An electrostatic interaction between BlpC and BlpH dictates pheromone specificity in the control of bacteriocin production and immunity in *Streptococcus pneumoniae J. Bacteriol.* **197** 1236–48

[59] Lyon G J, Wright J S, Muir T W and Novick R P 2002 Key determinants of receptor activation in the agr autoinducing peptides of *Staphylococcus aureus Biochemistry* **41** 10095–104

[60] Aleta A and Moreno Y 2019 Multilayer networks in a nutshell *Annu. Rev. Condens. Matter Phys.* **10** 45–62

[61] Henke J M and Bassler B L 2004 Three parallel quorum-sensing systems regulate gene expression in Vibrio harveyi *J. Bacteriol.* **186** 6902–14

[62] Zitnik M and Leskovec J 2017 Predicting multicellular function through multi-layer tissue networks *Bioinformatics* **33** i190–8

[63] Shinde P and Jalan S 2015 A multilayer protein–protein interaction network analysis of different life stages in *Caenorhabditis elegans Europhys. Lett.* **112** 58001

[64] Rai A, Pradhan P, Nagraj J, Lohitesh K, Chowdhury R and Jalan S 2017 Understanding cancer complexome using networks, spectral graph theory and multilayer framework *Sci. Rep.* **7** 41676

[65] De Domenico M, Sasai S and Arenas A 2016 Mapping multiplex hubs in human functional brain networks *Front. Neurosci.* **10** 326

[66] De Domenico M 2017 Multilayer modeling and analysis of human brain networks *GigaScience* **6** gix004

[67] How B 1996 *Nature Works* (New York: The Science of Self-Organized Criticality Copernicus)

[68] Berg H C and Purcell E M 1977 Physics of chemoreception *Biophys. J.* **20** 193–219

[69] Mehta P and Schwab D J 2012 Energetic costs of cellular computation *Proc. Natl Acad. Sci.* **109** 17978

[70] Ruparell A, Dubern J F, Ortori C A, Harrison F, Halliday N M, Emtage A *et al* 2016 The fitness burden imposed by synthesising quorum sensing signals *Sci. Rep.* **6** 33101

[71] Schuster S, Kreft J-U, Brenner N, Wessely F, Theißen G, Ruppin E and Schroeter A 2010 Cooperation and cheating in microbial exoenzyme production—theoretical analysis for biotechnological applications *Biotechnol. J.* **5** 751–8

[72] Schaefer A L, Oda Y, Coutinho B G, Pelletier D A, Weiburg J, Venturi V, Greenberg E P and Harwood C S 2016 A LuxR homolog in a cottonwood tree endophyte that activates gene expression in response to a plant signal or specific peptides *mBio* **7** e01101 e01116

[73] Whiteley M, Diggle S P and Greenberg E P 2017 Progress in and promise of bacterial quorum sensing research *Nature* **551** 313–20

[74] Drescher K, Nadell C D, Stone H A, Wingreen N S and Bassler B L 2014 Solutions to the public goods dilemma in bacterial biofilms *Curr. Biol.* **24** 50–5

[75] Pai A, Tanouchi Y and You L 2012 Optimality and robustness in quorum sensing (QS)-mediated regulation of a costly public good enzyme *Proc. Natl Acad. Sci.* **109** 19810

[76] Waltermann C and Klipp E 2011 Information theory based approaches to cellular signaling *Biochim. Biophys. Acta, Gen. Subj.* **1810** 924–32

[77] Mousavian Z, Díaz J and Masoudi-Nejad A 2016 Information theory in systems biology. Part II: protein–protein interaction and signaling networks *Semin. Cell Dev. Biol.* **51** 14–23

[78] Suderman R and Deeds E J 2018 Intrinsic limits of information transmission in biochemical signalling motifs *Interface Focus* **8** 20180039

[79] Chu D, Prokopenko M and Ray J C J 2018 Computation by natural systems *Interface Focus* **8** 20180058

[80] Rodrigo G and Poyatos J F 2016 Genetic redundancies enhance information transfer in noisy regulatory circuits *PLoS Comput. Biol.* **12** e1005156

[81] Mehta P, Goyal S, Long T, Bassler B L and Wingreen N S 2009 Information processing and signal integration in bacterial quorum sensing *Mol. Syst. Biol.* **5** 325

[82] Friedman J, Higgins L M and Gore J 2017 Community structure follows simple assembly rules in microbial microcosms *Nat. Ecol. Evol.* **1** 0109

[83] Sumner E R and Avery S V 2002 Phenotypic heterogeneity: differential stress resistance among individual cells of the yeast *Saccharomyces cerevisiae Microbiology* **148** 345–51

[84] Long T, Tu K C, Wang Y, Mehta P, Ong N P, Bassler B L and Wingreen N S 2009 Quantifying the integration of quorum-sensing signals with single-cell resolution *PLoS Biol.* **7** e1000068

[85] Anetzberger C, Pirch T and Jung K 2009 Heterogeneity in quorum sensing-regulated bioluminescence of Vibrio harveyi *Mol. Microbiol.* **73** 267–77

[86] Pérez P D and Hagen S J 2010 Heterogeneous response to a quorum-sensing signal in the luminescence of individual Vibrio fischeri *PloS One* **5** e15473

[87] Garmyn D, Gal L, Briandet R, Guilbaud M, Lemaître J-P, Hartmann A and Piveteau P 2011 Evidence of autoinduction heterogeneity via expression of the Agr system of Listeria monocytogenes at the single-cell level *Appl. Environ. Microbiol.* **77** 6286–9

[88] Grote J *et al* 2014 Evidence of autoinducer-dependent and -independent heterogeneous gene expression in Sinorhizobium fredii NGR234 *Appl. Environ. Microbiol.* **80** 5572–82

[89] Feltner J B *et al* 2016 LasR variant cystic fibrosis isolates reveal an adaptable quorum-sensing hierarchy in *Pseudomonas aeruginosa mBio* **7** e01513 e01516

[90] Guo X, Silva K P T and Boedicker J Q 2019 Single-cell variability of growth interactions within a two-species bacterial community *Phys. Biol.* **16** 036001

[91] Guo X and Boedicker J Q 2016 The contribution of high-order metabolic interactions to the global activity of a four-species microbial community *PLoS Comput. Biol.* **12** e1005079

[92] Razo-Mejia M and Phillips R 2019 First-principles prediction of the information processing capacity of a simple genetic circuit (bioRxiv:594325)

[93] Larkin J W *et al* 2018 Signal percolation within a bacterial community *Cell Syst.* **7** 137–45

[94] Silva K P T, Yusufaly T I, Chellamuthu P and Boedicker J Q 2019 Disruption of microbial communication yields a two-dimensional percolation transition *Phys. Rev.* E **99** 042409

[95] Ratzke C and Gore J 2016 Self-organized patchiness facilitates survival in a cooperatively growing Bacillus subtilis population *Nat. Microbiol.* **1** 16022

[96] Kim H J, Boedicker J Q, Choi J W and Ismagilov R F 2008 Defined spatial structure stabilizes a synthetic multispecies bacterial community *Proc. Natl Acad. Sci.* **105** 18188

[97] Nadell C D, Drescher K and Foster K R 2016 Spatial structure, cooperation and competition in biofilms *Nat. Rev. Microbiol.* **14** 589–600

[98] Borer B, Tecon R and Or D 2018 Spatial organization of bacterial populations in response to oxygen and carbon counter-gradients in pore networks *Nat. Commun.* **9** 769

[99] Ebrahimi A, Schwartzman J and Cordero O X 2019 Cooperation and spatial self-organization determine rate and efficiency of particulate organic matter degradation in marine bacteria *Proc. Natl Acad. Sci. USA* **116** 23309

[100] Momeni B, Waite A J and Shou W 2013 Spatial self-organization favors heterotypic cooperation over cheating *eLife* **2** e00960

[101] Haagensen J A J, Hansen S K, Christensen B B, Pamp S J and Molin S 2015 Development of spatial distribution patterns by biofilm cells *Appl. Environ. Microbiol.* **81** 6120

[102] Chandrasekaran K and Bose B 2019 Percolation in a reduced equilibrium model of planar cell polarity *Phys. Rev.* E **100** 032408

[103] Essam J W 1980 Percolation theory *Rep. Prog. Phys.* **43** 833–912

[104] Chen A Y, Deng Z, Billings A N, Seker U O S, Lu M Y, Citorik R J, Zakeri B and Lu T K 2014 Synthesis and patterning of tunable multiscale materials with engineered cells *Nat. Mater.* **13** 515–23

[105] Hong S H, Hegde M, Kim J, Wang X, Jayaraman A and Wood T K 2012 Synthetic quorum-sensing circuit to control consortial biofilm formation and dispersal in a microfluidic device *Nat. Commun.* **3** 613

[106] Brenner K and Arnold F H 2011 Self-organization, layered structure, and aggregation enhance persistence of a synthetic biofilm consortium *PloS One* **6** e16791

[107] Choudhary S and Schmidt-Dannert C 2010 Applications of quorum sensing in biotechnology *Appl. Microbiol. Biotechnol.* **86** 1267–79

[108] Jiang Q, Chen J, Yang C, Yin Y and Yao K 2019 Quorum sensing: a prospective therapeutic target for bacterial diseases *BioMed Res. Int.* **2019** 2015978

[109] Hsu R H, Clark R L, Tan J W, Ahn J C, Gupta S, Romero P A and Venturelli O S 2019 Microbial interaction network inference in microfluidic droplets *Cell Syst.* **9** 229–42

[110] Ziesack M *et al* 2019 Engineered interspecies amino acid cross-feeding increases population evenness in a synthetic bacterial consortium *mSystems* **4** e00352 e00319

[111] Goldford J E, Lu N, Bajić D, Estrela S, Tikhonov M, Sanchez-Gorostiaga A, Segrè D, Mehta P and Sanchez A 2018 Emergent simplicity in microbial community assembly *Science* **361** 469–74