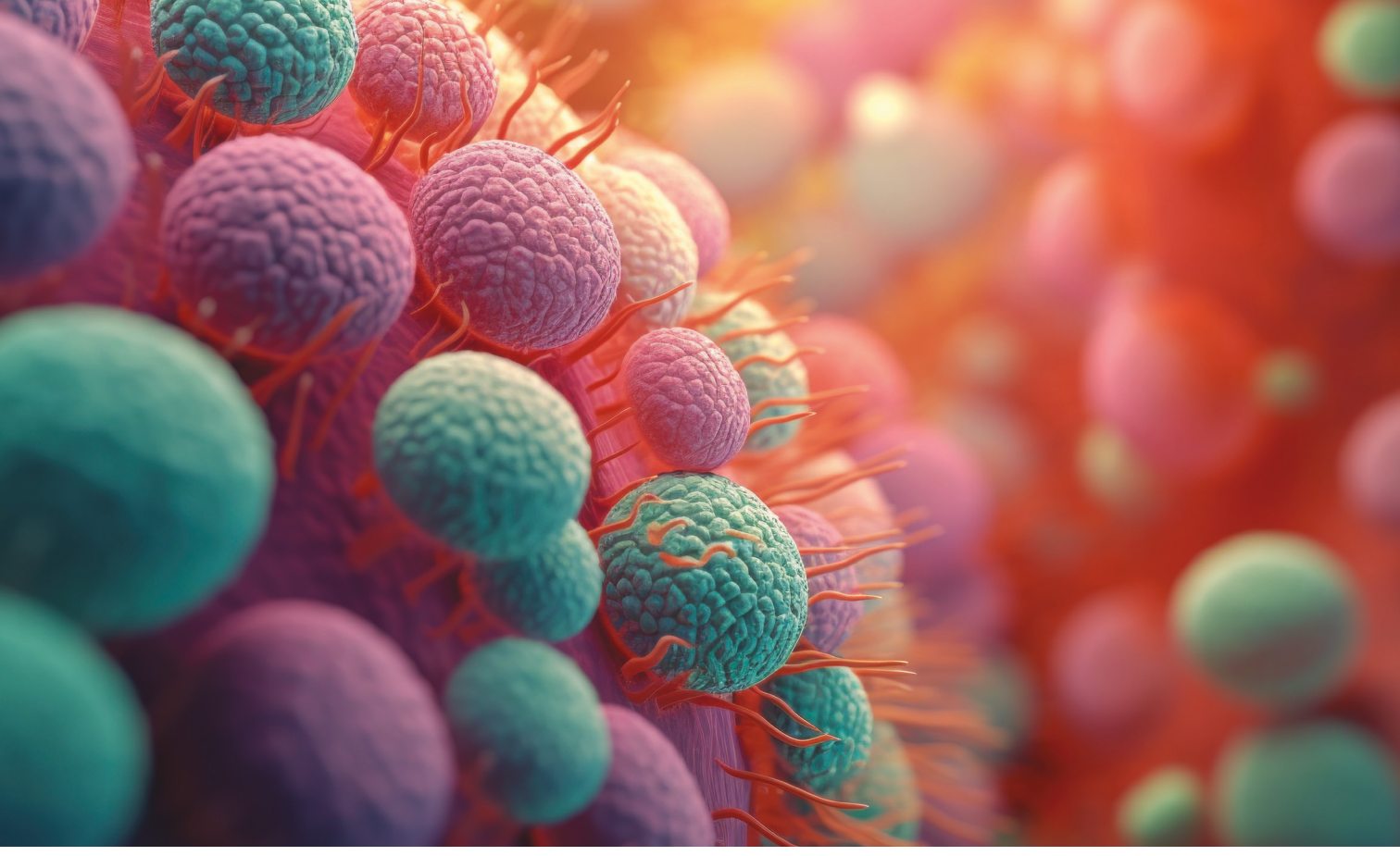


IMAGE LICENSED BY INGRAM PUBLISHING

Microbes as Communication & Decision-Making Networked Communities

MICROBIAL COMMUNITIES EXHIBIT complex behavior and play significant roles in many biological phenomena. Understanding the communication within and between bacterial species can illuminate the *how*, as well as the *why* of numerous interactions that enable their collective behavior. In this position paper, we first discuss bacterial molecular communication in multihop settings. We address this concept in the context of local and global quorum sensing (QS) within a colony and then consider induced QS at a distance between different, spatially separated communities. We then investigate *how* the information is shared between cells when forming

Digital Object Identifier 10.1109/MNANO.2023.3262431
Date of current version: 31 May 2023

JONI SHASKA, MUSTAFA CAN GURSOY, TYLER D. ROSS, YU-YU CHENG, ADAM KRIEGER, MARGARET THAIRU, OPHELIA S. VENTURELLI, JAMES BOEDICKER, JO HANDELSMAN, AND URBASHI MITRA

1932-4510/12/23©2023IEEE

a quorum, particularly the intertwined relationship between cells' observations, actions, and the state of their surrounding environment. Lastly, we extend to multi-species systems where all species coexist and interact, leveraging concepts from multiple-input-multiple-output (MIMO) communications. Community robustness and resiliency is also explored.

Bacteria are single-celled organisms that constitute some of the earliest forms of life (over three billion years old) and have an aggregate biomass that is at least an order of magnitude larger than all animals combined.

INTRODUCTION

Bacteria are single-celled organisms that constitute some of the earliest forms of life (over three billion years old) [1] and have an aggregate biomass that is at least an order of magnitude larger than all animals combined [2]. Although bacteria are considered one of the simplest forms of life, they typically live in communities that contain hundreds or thousands of species, which represent a genetic complexity 1000-fold greater than the human genome. These microbial communities are essential to all life on Earth. They direct biogeochemical cycles that influence climate change, soil health, and water quality. Microbial communities determine the health of their plant and animal hosts (e.g., gut biome) and are responsible for food fermentation. Accurate modeling of bacterial populations could enable the design of efficient microbial fuel cells or bacterial infection prevention without the need for antibiotics [3].

Interactions among members of bacterial communities are governed by the interchange of chemicals, including small organic molecules and ions. From a communications engineering perspective, these interactions can be considered as examples of *molecular communication (MC)* [4], [5], which refers to natural or synthetic systems that convey information using chemical signals. As an example, one outcome of signaling can be the formation of a *quorum* in which a community expresses new genes which enable new collective behavior [6], [7]. Inspired by recent theoretical advancements in modeling bacterial MC [8], [9], [10], as well as experimental studies to exploit and engineer microbial interactions [11], [12], [13], this position paper

1) presents theoretical abstractions of modeling cell- or colony-level bacterial communities,
2) introduces new experimental setups for spatially separated bacterial communication systems,
3) and discusses relevant applications of these methods to study communities.

The rest of the paper is organized as follows: The "Multihopped Signaling" Section discusses spatially constrained and unconstrained settings that leverage multihop MC among bacterial colonies to induce quorum sensing at a distance. The "Many-to-One Networks" Section considers microbial communities with a different underlying topology; in particular, from a modeling perspective, many-to-one networks are considered and the community is considered to be a decentralized decision-making system. The "Multi-Species Communities" Section focuses on populations with heterogeneous species or strains and by grouping each species into a virtual user, maps the signaling framework to that of a multi-input/multi-output (MIMO) communication channel. The "Conclusion" Section concludes the paper. Within each section, we specifically examine how these communities engage in actions to promote resiliency of the community.

MULTIHOPPED SIGNALING

Communication systems in which signals are transmitted via *repeaters*, or multihopped communications, have been continually studied since the 1950s. A seminal piece of work in this area is the PhD dissertation of Charles Desoer [14], whose analysis of *cascaded channels* includes the result that the capacity of a multi-hopped channel is that of the worst-case single-hop link. Microbial systems, as well as engineered wireless systems, use multi-hopped communications to overcome a common challenge: signal degradation. Many microbes employ the diffusion of molecules to signal. Each emitted molecule exhibits Brownian motion in the channel [4], which causes its arrival time at the receiver to be stochastic [15]. Molecular exchange via diffusion is limited by the scaling of distance traveled over time, with distance proportional to time squared. Diffusive signal exchange becomes ineffective over reasonable times when the colonies are separated by several millimeters. Finally, in the parlance of communications engineering, diffusive communication channels experience inter-symbol interference, much like their radio-based counterparts.

In this section, we examine microbial multi-hopped communications for inducing quorum sensing across distance. Experiments were applied in the context of free-space interaction as well as the more spatially constrained environments of microfluidics devices. There are some key contrasts in signaling mechanisms to consider when comparing wireless to microbial repeater systems: positive feedback, leakage, and relatively longer processing delays at each node due to slow biochemical reactions. A key distinguishing property of many of the microbial strains we have investigated is the ability to achieve a "quorum." In quorum sensing, when the concentration of a compound or molecule produced by the bacteria within the population exceeds a threshold, the bacteria express a suite of genes leading to new population behavior such as luminescence or infection of a host [6], [7]. With sufficient such expression, the community achieves a *quorum*.

A question of interest is whether one can interpret these vesicles as packets in a communications system which typically employ local error correction to ensure the fidelity of the packet.

UNCONSTRAINED MULTIHOPPED MICROBIAL SIGNALING

The main mechanism exploited by microbial colony repeaters (i.e., relay nodes) when inducing quorum sensing over a distance is positive feedback of signal (molecule) production. Cells in environments with low signal concentration produce signal at a low, basal rate. However, as signal accumulates to higher concentrations, eventually exceeding the threshold concentration for cells to strongly detect and respond to the signal, the rate of signal production increases for each "activated" cell. This positive feedback on signal production enables cells to coordinate activity over distances much longer than the length scale of diffusion [16]. This way, cells in an adjacent colony, once activated, amplify the signal production, allowing the now combined signaling gradient from both colonies to extend further at shorter times.

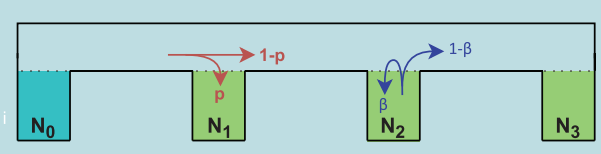
This concept of spatially separated colonies increasing the rate at which signal spreads over long distances was demonstrated in numerical simulations of quorum sensing [17]. We deem this environment as *unconstrained* as the locations of the bacteria were not specified. This will be in contrast to the microfluidic relay system we discuss in the next subsection. As a large single colony of cells was broken up into multiple smaller colonies dispersed at random positions, the region of space with concentration of signal that is above the threshold significantly increased. As the colonies grew smaller and more dispersed, activation at each colony involved signal from multiple adjacent colonies combining to exceed the signal threshold concentrations. This transition from *local quorum sensing* to *global quorum sensing* enables small, adjacent colonies to activate over large spatial ranges. We observe that the ability to transition imbues the population with robustness as the colony does not need to be uniformly and densely spaced to achieve a quorum.

The method of signal delivery may influence the spatial distance and dynamics of signal exchange and quorum sensing activation [18]. Recent work has shown that signal is not only exchanged by release of individual molecules into the environment, but that signal can also be packaged into and exchanged via extracellular vesicles. Bacterial extracellular vesicles are thought to be produced by all bacteria and are approximately $100\,\text{nm}$ in diameter [19]. Upon formation, the molecular composition of vesicles includes bacterial membrane, proteins, genetic material, and other molecules from inside the cell [20]. Many signaling molecules, especially hydrophobic signals that tend to partition into membranes, are often incorporated into vesicles. Combining signal-loaded vesicles with cells can lead to quorum sensing activation, as vesicles create a chemical environment separated from external conditions, potentially protecting signal from degradation during transport. For environments with high rates of signal degradation, free signals can only be exchanged over short distances. Vesicles protect signals and enable long distance exchange. A question of interest is whether one can interpret these vesicles as packets in a communications system which typically employ local error correction to ensure the fidelity of the packet.

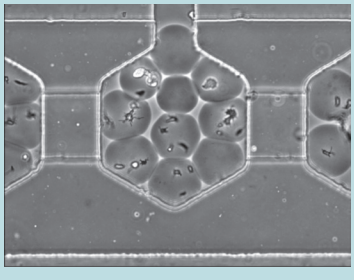
A second advantage of loading signal in vesicles is that the vesicle potentially keeps packets of signal together during transport. Free signal spreads out during transport, but a diffusing vesicle could maintain a constant signal cargo. Vesicles made in locations with high signal concentrations would make vesicles loaded with a high concentration of signal. These vesicles can then diffuse and deliver concentrated packets of signal to distant cells. Given that threshold concentrations of signal are often only $10-100$ molecules of signal per cell, long distance delivery of a single vesicle could result in quorum-sensing activation in the receiving cell.

CONSTRAINED MICROFLUIDIC MICROBIAL RELAY SYSTEMS

In this subsection, we consider a more controlled environment wherein microbial repeater communities are isolated via microfluidic systems. An important feature is that the distance between



(a) Depiction of a 3-hop bacterial relay theoretical system model.



(b) A node in an n-hop experimental relay device.

FIGURE 1 A linear and multihop MC system of spatially separated bacterial communities.

microbes (or collections of microbes) is pre-determined. Modeling of such systems using stochastic theory was undertaken in [21] using classical queuing theory. In particular, electron transfer between bacteria in cables was modeled by developing queueing models for biological processes such as adenosine triphosphate (ATP) production. These models were further leveraged to compute the channel capacity of such a system in [22].

In our previous system design (FAIRY [13]), each node of the relay was constructed as a spatially distinct chamber containing immobilized populations of bacteria. The initial node contained a transmitter (sender) microbial strain while the rest contained a receiver (repeater) strain. Communication between nodes was achieved through metabolic production and exchange of an acyl homoserine lactone (AHL), which is a microbial quorum sensing molecule. Briefly, both strains harbor an inducible promoter that regulates production of AHL. Induction within the sender is driven by arabinose (a small molecule), while induction within the repeater is driven by AHL. In addition to the input signal intensity, the output AHL level of a node is also a function of its colony fitness and population size [23], [24]. Furthermore, AHL being both the input and output of the repeater strain leads to a **positive self-feedback loop** of AHL production. The positive feedback loop serves to amplify the traversing signal and promote switch-like activation of repeater nodes. Since the system used in [13] was closed, i.e., batch culture, propagation of the signal proceeds until the cells exhaust their energy supply.

A challenge in the modeling and analysis of microbial signaling systems is understanding what the metric of interest should be. Providing quantitative measures for "biological fitness," for example, has been elusive. To this end, we assumed that minimizing the end-to-end delay for activation, T_a, would be a goal of interest in the microbial relay system. In our prior work [10], we provided a model for bacterial relay signaling for an n -hop system. Therein, T_a is defined as the time between the initial stimulus of the transmitter node (N_0) and the activation time of the receiver node (N_{n-1}) and is considered as a metric to be optimized. The work considers a 1D diffusive channel where there is transmitter (TX), receiver (RX), and $n-1$ relay nodes, with all nodes as fully passive receivers [15]. The positive self-feedback is characterized by a parameter β, which denotes the portion of the signal at each node being trapped by the node itself. A representative figure of an expanded version of the model considered in [10] is presented in Figure 1(a).

In this subsection, we focus on a novel colony-based relay system, which we have devised in a microfluidic format. Henceforth, we refer to our setup herein as Signal Propagation Across Cellular Encapsulations (SPACE). The goal of SPACE is to permit relay signaling on time scales longer than typical batch culture allows. To accomplish this, we implemented SPACE in a traditional flow-cell style, where perfusion of fresh media promotes continuous growth of the colonies. A challenge associated with spatial patterning of microbes in flow cell designs is fabrication of cellular barriers that are permissive to molecular exchange. Since the effective size of a bacterial cell is approximately 1 μm, these barrier features must be fabricated on a sub-micron scale. While possible, fabrication at this scale requires high performance equipment that is not available to most. Our approach to solving this problem was to encapsulate cells in hydrogel beads, which increases the size of the working biological unit from a single micron to tens of microns. A simple channel connecting adjacent nodes can then be designed to exclude the much larger beads rather than individual cells. This can be achieved when the minimum dimension of the channel opening is roughly no greater than half of the bead diameter. For our system, we used a bead diameter of 70 μm and a diffusion channel depth of 5 μm.

In contrast with batch culture relay systems, flow cell microfluidics permits continuous microbial growth through perfusion of rich media, as well as temporal control over input signals.

In contrast with batch culture relay systems, flow cell microfluidics permits continuous microbial growth through perfusion of rich media, as well as temporal control over input signals. In addition to our considerations herein, we note that these benefits could support additional studies where bits of information are transmitted by periodically switching the sender on and off, equivalent to the concentration shift keying (CSK) modulation commonly used in MC [5]. In such a setup (with CSK), as each relay node would introduce an additional variance when processing its input signal, a relay-aided bacterial MC setup is potentially prone to error propagation from one hop to the next. Furthermore, previous work suggests that the signal-to-noise ratio actually improves with distance above some critical input frequency [11] (potentially due to the frequency domains of intra- and extracellular noise). These two phenomena jointly imply that, contrary to intuition, utilizing relay nodes for high-data rate bacterial MC systems may actually hurt communication fidelity. This gives rise to an interesting trade-off between fidelity and delay and suggests that the number of relays might be an optimization variable in device design. We pose designing low error rate digital MC systems under such a constraint as a possible open research direction, and focus solely on end-to-end delay as the metric of interest in the remainder of the subsection.

In [10], the self-feedback-induced signal intensity increase is modeled to halt at a certain maximum saturation limit. When modeling delay associated with SPACE, we follow a purely data-driven

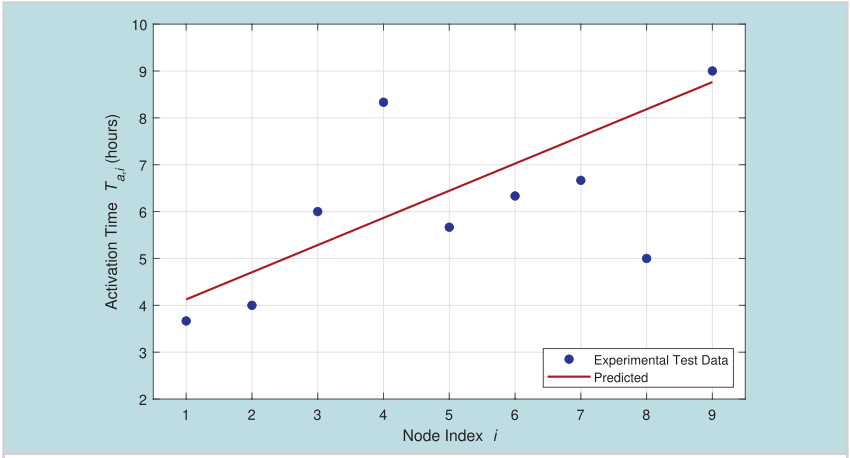


FIGURE 2 Regression for a 10-hop relay system using (1) on the SPACE device (see Figure 1).

approach herein (as opposed to a parametric characterization done in [10]), thus imposing no such hard limit.[1] Furthermore, in [10], each colony is modeled as fully passive receivers. However, in wet-lab experimental setups where the majority of the chamber is filled with bacteria such as in SPACE (see Figure 1(b)), the physical obstruction caused by the cells and their surroundings call for modeling the receivers as partially absorbing entities with **leakage**. Thus, a portion of the signal might still permeate the colony in our model herein, which is denoted by $(1-p)$ in the model of Figure 1(a).

With a fully absorbing receiver ($p=1$), and with the assumption that each link was identical, the end-to-end delay is expected to be well approximated as

$$T_a \approx b + n \times T, \tag{1}$$

where T is the single hop delay, and b captures the initial delay due to metabolic adaptation of the colonies. Note that (1) can be written for each hop's activation time as $T_{a,i} \approx b + i \times T$ which is affine in i and allows for a linear regression on experimental data to fit for b and T, which we will do in the sequel. Generalizing the argument for $p < 1$, $T_{a,i}$ would be different than that for $p=1$, as i) the leaked/permeated signal reduces input to a colony, but ii) the leaked signal propagates towards the next colony, starting its activation. This phenomenon can be accounted for by incorporating an additional, learnable function $f(i)$, yielding

$$T_a = T_{a,n} \approx b + n \times T + f(n). \tag{2}$$

Note that the leaked signal's decrease in node i would be expected to be larger than the increase at node $i+1$, as the latter also necessitates diffusive propagation and absorption at node $i+1$. Thus, imperfect absorption and non-zero permeability is expected to increase delays, and $f(i)$ is expected to be positive for $p<1$.

Herein, we focus on the particular case, where $f(i)=0$ in (2), and regress for b and T over three sets of experiments on the described SPACE setup with $n=10$. Two out of three experiments were used for training for b and T, whereas the third one is used for testing. Each node's readout (GFP intensity, as a surrogate for AHL production) is normalized with respect to its own maximum value, and $T_{a,i}$ is selected as the time it takes to reach half-maximum [13]. The overall results of Figure 2 suggests that setting $f(i) \approx 0$ provides reasonable accuracy, given the modest amount of experimental data. In addition to providing tractability, this phenomenon implies that considering each colony as a fully absorbing entity is a plausible consideration for the system of interest.

ON THE RESILIENCE OF MOLECULE PROCESSING MICROBIAL NODES

As also mentioned under the self-feedback discussion in prior sections, microbes exhibit tight regulation over catabolic pathways to avoid extraneous resource allocation and metabolic burden [25], [26]. This can be a result of negative self-feedback loops that regulate output intensity, as well as changes in gene expressions [27], [28], [29]. Our prior work [30] models each node as a molecule "processing" unit, akin to a packet/job scheduler in a wireless communication/resource allocation system. The theoretical findings in [30] suggest that under temporally varying input signal conditions, such negative feedback-induced output intensity balancing can indeed bring significant improvement in energy cost reduction and improve fitness, in cases with or without signal loss due to molecular degradation [11], [31]. In particular, in cases where increasing output intensity yields a convex increase in metabolic energy cost, balancing the output rate as much as possible is the provably optimal strategy. That said, the theoretical findings of [30] rely on non-causal information on arrival intensity, which limits its one-to-one applicability to biological settings. Our future path therein includes alleviating this assumption, as well as introducing additional bio-compatible costs and constraints to proceed our model towards more accurately abstracting real-time bacterial relay communication.

MANY-TO-ONE NETWORKS

Many-to-one communication and signaling systems are commonplace in wireless communications as seen in sensor networks, multiple-access channels, and many Internet-of-Things deployments. It is natural to consider such a framework for modeling microbial systems with some key differences. The environmental state, which can be more complex than simply the interference present, strongly influences microbial behavior. As such, there is significant coupling in microbial systems as they interact and change their environment. Such feedback mechanisms must be carefully described. The engine of such coupling can often be captured via signaling. Message exchange between nodes is also a feature of engineered networks, with consensus signaling in an *ad hoc* network as a prime example. However, in such systems, signal mixing

[1]However, it should be noted that in experimental settings, it is expected that colonies may exhibit a similar self-regulation to avoid extra metabolic burden of over-production.

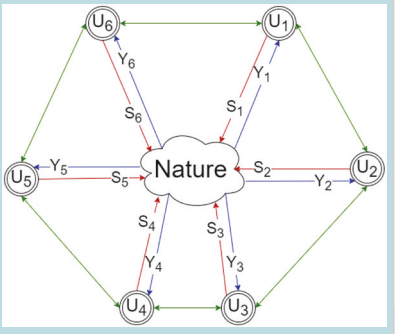


FIGURE 3 Depiction of coupling model for a decision-making microbial network.

A feature to integrate in the future is that of time-varying network topologies; these have been considered in wireless sensor networks, the rate of change for biological systems is different.

is intentional in order to learn a global function of the network. Elucidating the behaviors or states of individual bacteria in a microbial community from mixed signals is much more challenging. An opportunity that arises from mixed signaling is learning information about the entire community or a neighborhood of bacteria.

A feature to integrate in the future is that of time-varying network topologies; these have been considered in wireless sensor networks, the rate of change for biological systems is different. There is limited mobility, but perhaps more distinctive is the loss of "agents" as bacteria do die. Thus, the number of nodes/bacteria in a network is governed by a birth-death process. Several modern networks of interest share this phenomenon, e.g. social networks and other human-decision-making networks. The system model of Figure 3, endeavors to capture these features through the notion of agent and network states.

COLONIES AS LEARNING ENVIRONMENTS

In our modeling we consider an omniscient agent receiving the signaling from multiple decentralized agents. We have also considered more general scenarios without the centralized agent. In particular, population growth in quorum sensing is modeled using the queuing approach of [21] in [3]. Therein, there is an excellent match between predicted population sizes and experimental data. However, a challenge is that the decision-making of each agent is not explicitly considered. A control and decision-theoretic approach is considered in [32] for the discrete-time case. It can be shown that for noiseless observations, the optimal decision structure is threshold based (an individual cell expresses new genes when the level of autoinducer in the environment exceeds a threshold). Moreover, building off of the model presented in [3], the observation (autoinducer molecules) seen by an individual cell is modeled as a Poisson random variable

$$P_X(x) = \frac{\lambda^x \exp -\lambda}{x!} \tag{3}$$

with parameter $\lambda > 0$. Then, the work in [32] finds the optimal activation thresholds that maximize the colony fitness and verifies the theoretical findings with experimental data. In [12], the problem is framed as a continuous-time sequential optimization problem where each cell in the colony decides at each time instant whether to activate – activation is costly – in order to maximize the future population size. A surprising observation from our analysis is that the community must consider both current and future payoffs. This observation is also reflected in the experimental data which is well modeled by our theoretical approach. However, these models still lack a detailed interaction model to capture the coupling between the agents/bacteria and the environment.

Thus, our recent focus has been on capturing this coupling, which will also impact the analysis of social learning mechanisms. In particular, previous decentralized inference theory adopts a key conditional independence assumption for tractabililty without the assumption, determining optimal detection rules becomes NP-hard. Denote U_i as cell i's behavior and H as the true state of nature. We have the following coupled equations:

$$H = g(U_1, U_2, \ldots, U_n)$$
$$U_i = f(U_1, U_2, \ldots, U_n, H) \neq f(H)$$

The inequality shows the resulting decoupling if the conditional independence assumption is invoked. We have shown [33] how to obviate this strong assumption through the introduction of the notion of a cell's state, X_i which acts as a "summary" for the behavior of the other cells and circumvents the NP-hardness of microbial interactions.

$$U_i = f(U_1, U_2, \ldots, U_n, H) = f(X_i, H). \tag{4}$$

In [33], the asymptotic learning rate is characterized, and it shown that each agent employing a common decision strategy is also asymptotically optimal (although not optimal in the small number of cells regime). The ability to consider a common strategy across all bacteria significantly simplifies the analysis of asymptotically large colonies. In particular, [33] provides an important first step towards the analysis of multi-species colonies where the interaction models are species-dependent.

ON THE RESILIENCE OF MICROBIAL NETWORKS

While microbial systems are inherently complex, we see that stochastic methods have the capability of capturing intricate coupled behavior. In particular, stochastic modeling can capture the effects of variations across cells as well as unmodeled effects that can be appropriate as noise. Particular to the decentralized agent modeling, we see that threshold-based decision rules which have been the conventional wisdom for such behaviors as quorum sensing can be mathematically justified [32] motivating our

> Communities appear to self-regulate to limit outlier behavior as that it is in the community's longer term best interests.

current framework. Our ultimate goal is to understand the optimal trade-off between population growth and colony fitness and to leverage experimental outcomes to enable the design and understanding of such microbial communities. We see from [33] and [34] that there are optimal operating regimes which suggest that inherent self-regulation is beneficial to the colony. This self-regulation, to improve colony fitness, was observed experimentally in [12]. Furthermore, both [33] and [34] mathematically show that while individual decision rules for individual bacterium may be optimal for small populations, despite single-cell heterogeneity, common decision rules benefit the community.

Communities appear to self-regulate to limit outlier behavior as that it is in the community's longer term best interests. In contrast to many pure engineering system where "greedy is good," that is not necessarily what is seen in modeled systems that directly take biological constraints into consideration [12], [21].

MULTI-SPECIES COMMUNITIES

In the previous section, we focused on the signal coupling that is inherent to microbial signaling; herein, we introduce other unique features of microbial interaction which suggest that modified multiple-input, multiple-output (MIMO) models provide a useful perspective for analysis. We summarize recent developments in modeling microbial signaling through MIMO systems, as this area of research is rapidly expanding. Wireless MIMO systems have a rich history [35], [36] and have had broad impact in engineering applications. We have previously underscored the impact of signaling via diffusion which can introduce time-variation and uncertainty to timing as well as network topology. In our discussion of relay-systems we have also discussed the presence of self-interference and feedback. This feedback mechanism within a single cluster of cells can result in behavior that is seemingly independent of the overall colony as seen in recent experimental work [17]. The main reason why we will examine MIMO models in this context is to consider the self-signaling and to provide models which capture intra-species interaction in multi-species environments. Up to now, we have only considered single strains of bacteria. As will be seen, multi-species environments can lead to additional degrees of freedom when it comes to interaction and coupling, in particular, different strains can influence the transmitted signals of other strains in the community. Thus, new MIMO modeling and analysis is needed, as illustrated by the Microbial MIMO system given in Figure 4.

MICROBIAL SYSTEMS AS MIMO CHANNELS

Bacteria, as simple, single-cell organisms, can enable complex behaviors such as infection, bioluminescence, and biofilm production. Given the prevalence of bacteria and their role in animal and environmental health, it is important to understand these behaviors and the associated complicated interactions. These interactions are governed by signaling molecules and associated genetic expressions. Our prior models for quorum sensing and coupled interaction via a decentralized decision-making framework were in the context of a single species ("Many-to-One Networks" Section). We next generalize the problem environment by considering the presence of multiple bacterial strains, each capable of producing distinct autoinducer molecules. Considering heterogeneous species further challenges analysis due to increased coupling and interaction. Furthermore, each cell generates associated receptors for the different molecules. In

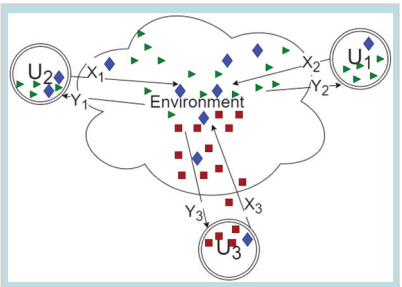


FIGURE 4 Microbial MIMO system. Cells send chemical signals into the environment and receive chemical signals consisting of a mixture of other cells' chemical signals.

fact, most quorum sensing signals interact with many receptors[2] and impact the downstream cellular response to the signal, a phenomenon commonly referred to as crosstalk [38]. Thus, in cross-talk, the wrong molecule binds to an unintended receptor. Crosstalk can have a stimulatory or inhibitory effect on cellular activation. The MIMO model enables the clean description of self-signaling in the multi-strain microbial environment.

MULTI-STRAIN/MULTI-SPECIES SIGNALING

Recently, in [39], an important conceptual advancement in the understanding of microbial quorum sensing networks is made by demonstrating that quorum sensing networks can be represented as Hopfield networks [40]. Each strain corresponds to a particular node whose state represents whether the strain is activated or not. Each strain has its own autoinducer molecule. Activation is based on the multiple signaling inputs which are linearly combined with different weights and fed into a non-linear function (usually sigmoidal). Thus, each strain responds to all the different quorum-sensing molecules in the community as input. In [39], the optimal number of strains needed to maximize the capacity of the network as described by the Boltzmann entropy is determined. Furthermore, this number is consistent with patterns of diversification of the quorum sensing system of the microbial species Staphylococcus aureus, suggesting a possible selective

[2]Microbial cells can also express so-called, orphan receptors, for which the associated cognate signal has not been identified [37].

evolutionary constraint in quorum sensing networks.

The MIMO neural network model was further employed to study the heterogeneous spatial structure within a microbial community via reaction-diffusion models [17]. Colony dispersals are varied and it is shown that at low dispersal, which corresponds to a community state in which most cells are located in a small number of highly clustered colonies, the quorum sensing systems are dominated by the localized signal. However, as the community state transitions to higher dispersal, individual colonies are unable to self-activate and thereby become dependent on the global quorum sensing signal to active. The neural network model is used to explicitly explore crosstalk within a microbial community [41]. Five different strains of Bacillus subtilis were employed. Once the weights of activation/inhibition of each quorum sensing molecule were established for each node, the neural network model was used to accurately predict community-level signaling states in the community that are an integration of all signal present in the community at a given time point.

Cells that belong to different genotypes can interact with each other in highly complex ways. To illustrate this, a recent experiment arrayed colonies of different Bacillus species. Many of the Bacillus isolates are capable of producing biofilms. Bacillus can release compounds that inhibit the growth of other species. For example, B. subtilis (right colony) and B. mojavensis display a negative interaction. In comparison, some Bacillus strains are more 'friendly' towards other Bacillus such that two biofilms can merge. For example, B. atrophaeus and B. subtilis (right colony) display a neutral or potentially positive interaction. The presence of other species can also induce B. subtilis motility. In summary, interactions between different species of bacteria generate collective behaviors. The results of this experiment can be seen in Figure 5.

To begin modeling signaling among different species, we analyzed a model community, THOR, that includes three species that are normally found in close proximity to plant roots (the hitchikers of the rizhosphere, THOR) [42].

> Bacteria, as simple, single-cell organisms, can enable complex behaviors such as infection, bioluminescence, and biofilm production.

To accommodate this model system, we examined the scenario [34] where agents' observations depend not only on the underlying state of nature, but also on the empirical distribution of the genetic expressions of the colony. Hence, the statistical dependencies of agents' observations are global, as opposed to local in [33]. The model allows for agents in different classes to have distinct coupling with agents from another class, which is inherent to THOR. There are three strains of interest: *Flavobacterium*, *Bacillus*, and *Pseudomonas*. This model reduces the complexity of the natural community from over 1,000 species to three. Our work shows that the three species greatly affect each others' patterns of gene expression, including genes involved in the synthesis of signaling molecules. In particular, When grown in isolation, Bacillus produces a biofilm which can be modeled by the methods in [33]. When grown together with Flavobacterium, the Bacillus strain is eradicated by an antibiotic produced by the Flavobacterium, and no biofilm is produced. However, when all three strains coexist, Pseudomonas produces a chemical signal that shields Bacillus from Flavobacterium. In addition, not only is Bacillus protected from the harmful effects of Flavobacterium, but Bacillus produced more biofilm when all three strains are present. THOR contains a total of 17,000 genes and around 100 pathways that may generate signals. Through use of a mutant affected in one small molecule and genetic analysis, we found that one molecule was responsible for changes in the expression of thousands of genes in the other community members [43], [44]. A systematic genetic analysis of all pathways for synthesis of small molecules in all three members will generate a network model that accounts for the impact of each signal on gene expression in the other members.

Now, work is being done to bridge the gap between microbiology and experimental findings with statistical modeling. We have extended our

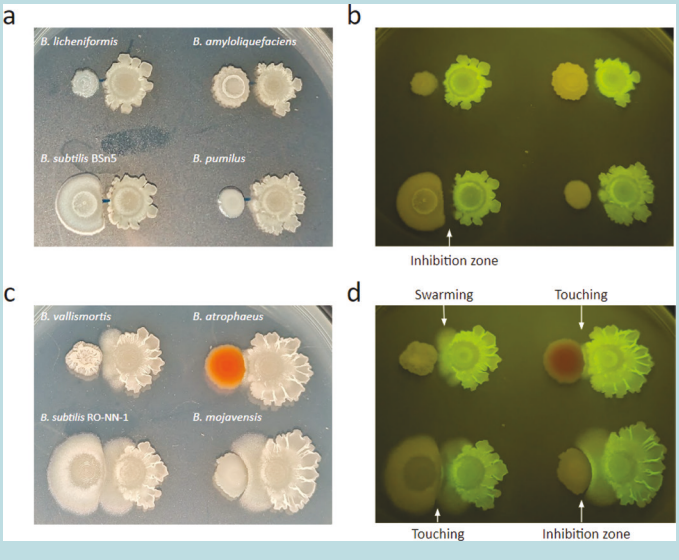


FIGURE 5 Biofilm interactions among the various Bacillus wild-type isolates.

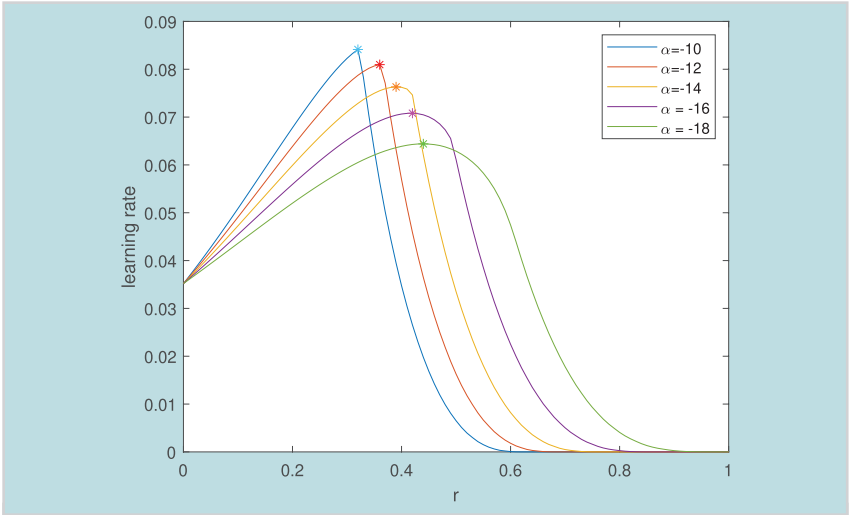


FIGURE 6 Community learning rate for a theoretical, two species microbial colony. Signal interference between species is measured by α. The ratio of population 1 to a fixed size for population 2 is described by r. The optimal ratio is indicated by a star.

prior [33] to the more general setting in [34]. Specifically, systems in which the behavior is governed by both the underlying hypothesis (individual state of the microbe), as well as an underlying empirical distribution on the network state (environmental state) is considered. Thus, there is significant coupling between the interim decisions of the agents and the signals they transmit. The performance of collective decision-making is asymptotically analyzed. Optimal ratios of species are computed in the context of both signal enhancement or jamming (as experienced in THOR). The results of the consideration of a two species scenario can be seen in Figure 6. The two species interact through the constant α, and the learning rate as a function of the population ratio for various values of α, as well as the optimal ratio are shown. These results show the sensitivity of not only the optimal ratio to α but also the functional dependence of the learning rate on population ratios. Currently, we are examining THOR data in order to tune and validate these models against experimental data. The goal is to determine signal parameter values and to evaluate the predictive value of the model.

MULTI-SPECIES COMMUNITY ROBUSTNESS

As seen in the multiple experimental results discussed, the interaction of multiple strains or species within a community leads to complex signaling and collective behaviors. The presence or absence of one strain/species can have an enormous impact. A goal of our engineering models is to determine optimal community operating points and see whether such operating points exist in nature or can be induced in engineered environments. The effectiveness of these analytical frameworks, such as the Hopfield network, to predict community behavior will have a significant impact on the understanding and design of microbial communities. Of particular interest is understanding what molecules and control mechanisms lead to microbial community resilience.

CONCLUSION

In this position paper, we examine microbial populations through the lens of signaling and sensing networks. The goal was to use communication, signal processing, and information theories to model and understand community behaviors. As the modeling efforts were strongly informed by microbiology and experimental findings, new experimental systems and data were presented. Theoretical abstractions to employ when modeling signaling and decision-making in bacterial communities were provided. In particular, we presented SPACE, a microfluidic-based multi-hop bacterial molecular communication testbed, and characterized the communication delay associated with it. In contrast to SPACE's spatially constrained structure, we discussed the effects of multihop signaling to achieve quicker quorum sensing in spatially "unconstrained" systems. Furthermore, we have explored the effects of significant coupling between cells in a microbial community and their surrounding environment, as well as coupling between cells, when reaching a common quorum decision akin to a decentralized detection system. Behaviors from microbial communities and interaction were presented and drove the modeling approach. Finally, we leverage the existing understanding on MIMO communication to describe microbial behavior in multi-species environments. As multi-species communities can engage in novel interactions and reflect real-environments, this scenario is of strong interest. The impact of different signaling molecules from different, heterogeneous species in a community environment is still not fully understood. The ultimate goal is to use modeling to better understand and potentially design or control microbial communities.

ACKNOWLEDGMENTS

This work was supported in part by ARO under Grant W911NF1910269, and in part by NSF under Grant CCF-2008927.

ABOUT THE AUTHORS

Joni Shaska (corresponding author: (shaska@usc.edu)) is with the Department of Electrical and Computer Engineering University of Southern California, Los Angeles, CA, 90007, USA.

Mustafa Can Gursoy (mgursoy@usc.edu) is with the Department of Electrical and Computer Engineering University of Southern California, Los Angeles, CA, 90007, USA.

Tyler D. Ross (tross5@wisc.edu) is with the Department of Biomedical Engineering University of Wisconsin-Madison, Madison, WI, 53706, USA.

Yu-Yu Cheng (ychen223@wisc.edu) is with the Department of Biochemistry University of Wisconsin-Madison, Madison, WI, 53706, USA.

Adam Krieger (adamkrie@usc.edu) is with the Department of Physics and

AstronomyUniversity of Southern California, Los Angeles, CA, 90007, USA.

Margaret Thairu (thairu@wisc.edu) is with the Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI, 53706, USA.

Ophelia S. Venturelli (venturelli@wisc.edu) is with the Department of Biomedical Engineering University of Wisconsin-Madison, Madison, WI, 53706, USA. and also with the Department of Biochemistry University of Wisconsin-Madison, Madison, WI, 53706, USA.

James Boedicker (boedicke@usc.edu) is with the Department of Physics and Astronomy University of Southern California, Los Angeles, CA, 90007, USA.

Jo Handelsman (jo.handelsman@wisc.edu) is with the Department of Plant Pathology University of Wisconsin-Madison, Madison, WI, 53706, USA.

Urbashi Mitra (ubli@usc.edu) is with the Department of Electrical and Computer Engineering University of Southern California, Los Angeles, CA, 90007, USA.

REFERENCES

[1] A. J. Kaufman, "Cyanobacteria at work," *Nature Geosci.*, vol. 7, no. 4, pp. 253–254, Mar. 2014.

[2] Y. M. Bar-On, R. Phillips, and R. Milo, "The biomass distribution on Earth," in *Proc. Nat. Acad. Sci.* vol. 115, no. 25, pp. 6506–6511, 2018.

[3] N. Michelusi, J. Boedicker, M. Y. El-Naggar, and U. Mitra, "Queuing models for abstracting interactions in bacterial communities," *IEEE J. Sel. Areas Commun.*, vol. 34, no. 3, pp. 584–599, Mar. 2016.

[4] T. Nakano, A. W. Eckford, and T. Haraguchi, *Molecular Communication*. Cambridge, U.K.: Cambridge Univ. Press, 2013.

[5] M. C. Gursoy, M. Nasiri-Kenari, and U. Mitra, "Towards high data-rate diffusive molecular communications: A review on performance enhancement strategies," *Digit. Signal Process.*, vol. 124, May 2022, Art. no. 103161.

[6] K. L. Visick and C. Fuqua, "Decoding microbial chatter: Cell-cell communication in bacteria," *J. Bacteriol.*, vol. 187, no. 16, pp. 5507–5519, 2005.

[7] S. Forst and K. Nealson, "Molecular biology of the symbiotic-pathogenic bacteria Xenorhabdus spp. and Photorhabdus spp," *Microbiological Rev.*, vol. 60, no. 1, pp. 21–43, 1996.

[8] A. Einolghozati, M. Sardari, and F. Fekri, "Design and analysis of wireless communication systems using diffusion-based molecular communication among bacteria," *IEEE Trans. Wireless Commun.*, vol. 12, no. 12, pp. 6096–6105, Dec. 2013.

[9] B. Krishnaswamy et al., "Time-elapse communication: Bacterial communication on a microfluidic chip," *IEEE Trans. Commun.*, vol. 61, no. 12, pp. 5139–5151, Dec. 2013.

[10] M. C. Gursoy, S. Gupta, O. S. Venturelli, and U. Mitra, "Optimizing the spatial topology of bacterial relay systems: Delay minimization," in *Proc. IEEE Int. Conf. Commun.*, 2022, pp. 1722–1727.

[11] S. Gupta, T. D. Ross, M. M. Gomez, J. L. Grant, P. A. Romero, and O. S. Venturelli, "Investigating the dynamics of microbial consortia in spatially structured environments," *Nature Commun.*, vol. 11, no. 1, May 2020, Art. no. 2418.

[12] M. S. Gangan, M. M. Vasconcelos, U. Mitra, O. C'mara, and J. Q. Boedicker, "Intertemporal trade-off between population growth rate and carrying capacity during public good production," *iScience*, vol. 25, no. 4, Apr. 2022, Art. no. 104117.

[13] S. Gupta, "Manipulating spatiotemporal variables to program coordinated behaviors in microbial consortia," Ph.D. dissertation, Univ. Wisconsin-Madison, Madison, WI, USA, 2021.

[14] C. A. Desoer, "Communication through channels in cascade," Ph.D. dissertation, Massachusetts Inst. Technol., Cambridge, MA, USA, 1953.

[15] V. Jamali, A. Ahmadzadeh, W. Wicke, A. Noel, and R. Schober, "Channel modeling for diffusive molecular communication–a tutorial review," *Proc. IEEE*, vol. 107, no. 7, pp. 1256–1301, Jul. 2019.

[16] J. K. Kim, Y. Chen, A. J. Hirning, R. N. Alnahhas, K. Josić, and M. R. Bennett, "Long-range temporal coordination of gene expression in synthetic microbial consortia," *Nature Chem. Biol.*, vol. 15, no. 11, pp. 1102–1109, 2019.

[17] T. I. Yusufaly and J. Q. Boedicker, "Spatial dispersal of bacterial colonies induces a dynamical transition from local to global quorum sensing," *Phys. Rev. E*, vol. 94, no. 6, 2016, Art. no. 062410.

[18] B. P. Weaver, C. A. Haselwandter, and J. Q. Boedicker, "Stochastic effects in bacterial communication mediated by extracellular vesicles," *Phys. Rev. E*, vol. 107, no. 2, 2016, Art. no. 024409.

[19] A. C. Cooke, A. V. Nello, R. K. Ernst, and J. W. Schertzer, "Analysis of Pseudomonas aeruginosa biofilm membrane vesicles supports multiple mechanisms of biogenesis," *PLoS One*, vol. 14, no. 2, 2019, Art. no. e0212275.

[20] H. M. McMillan and M. J. Kuehn, "The extracellular vesicle generation paradox: A bacterial point of view," *EMBO J.*, vol. 40, no. 21, 2021, Art. no. e108174.

[21] N. Michelusi, S. Pirbadian, M. Y. El-Naggar, and U. Mitra, "A stochastic model for electron transfer in bacterial cables," *IEEE J. Sel. Areas Commun.*, vol. 32, no. 12, pp. 2402–2416, Dec. 2014.

[22] N. Michelusi and U. Mitra, "Capacity of electron-based communication over bacterial cables: The full-CSI case," *IEEE Trans. Mol., Biol. Multi-Scale Commun.*, vol. 1, no. 1, pp. 62–75, Mar. 2015.

[23] J. Engebrecht, K. Nealson, and M. Silverman, "Bacterial bioluminescence: Isolation and genetic analysis of functions from vibrio fischeri," *Cell*, vol. 32, no. 3, pp. 773–781, 1983. [Online]. Available: https://www.sciencedirect.com/science/article/pii/0092867483900636

[24] P. K. Grant et al., "Orthogonal intercellular signaling for programmed spatial behavior," *Mol. Syst. Biol.*, vol. 12, no. 1, 2016, Art. no. 849, doi: 10.15252/msb.20156590.

[25] C. You et al., "Coordination of bacterial proteome with metabolism by cyclic AMP signalling," *Nature*, vol. 500, no. 7462, pp. 301–306, Aug. 2013, doi: 10.1038/nature12446.

[26] G. Wu, Q. Yan, J. A. Jones, Y. J. Tang, S. S. Fong, and M. A. Koffas, "Metabolic burden: Cornerstones in synthetic biology and metabolic engineering applications," *Trends Biotechnol.*, vol. 34, no. 8, pp. 652–664, Aug. 2016, doi: 10.1016/j.tibtech.2016.02.010.

[27] J. Cole, C. Olsson, J. Hershey, M. Grunberg-Manago, and M. Nomura, "Feedback regulation of rRNA synthesis in Escherichia Coli," *J. Mol. Biol.*, vol. 198, no. 3, pp. 383–392, Dec. 1987, doi: 10.1016/0022-2836(87)90288-9.

[28] S. Bhartiya, N. Chaudhary, K. Venkatesh, and F. J. Doyle, "Multiple feedback loop design in the tryptophan regulatory network of Escherichia coli suggests a paradigm for robust regulation of processes in series," *J. Roy. Soc. Interface*, vol. 3, no. 8, pp. 383–391, Nov. 2005, doi: 10.1098/rsif.2005.0103.

[29] M. C. Davis, C. A. Kesthely, E. A. Franklin, and S. R. MacLellan, "The essential activities of the bacterial sigma factor," *Can. J. Microbiol.*, vol. 63, no. 2, pp. 89–99, Feb. 2017, doi: 10.1139/cjm-2016-0576.

[30] M. C. Gursoy and U. Mitra, "Energy-Efficient Packet Scheduling under Two-Sided Delay Constraints," in *Proc. IEEE Int. Conf. Commun.* May 2023.

[31] A. Noel, K. C. Cheung, and R. Schober, "Improving receiver performance of diffusive molecular communication with enzymes," *IEEE Trans. Nanobiosci.*, vol. 13, no. 1, pp. 31–43, Mar. 2014.

[32] M. M. Vasconcelos, U. Mitra, O. Camara, K. P. Silva, and J. Boedicker, "Bacterial quorum sensing as a networked decision system," in *Proc. IEEE Int. Conf. Commun.*, 2018, pp. 1–6.

[33] J. Shaska and U. Mitra, "Decentralized decision making in multi-agent networks: The state-dependent case," in *Proc. IEEE Glob. Commun. Conf.*, 2021, pp. 1–6.

[34] J. Shaska and U. Mitra, "Type-senstive social learning," to be presented at the IEEE Int. Conf. Commun., 2023.

[35] G. J. Foschini, "Layered space-time architecture for wireless communication in a fading environment when using multi-element antennas," *Bell Labs Tech. J.*, vol. 1, no. 2, pp. 41–59, Autumn 1996.

[36] E. Telatar, "Capacity of multi-antenna Gaussian channels," *Eur. Trans. Telecommun.*, vol. 10, no. 6, pp. 585–595, 1999.

[37] V. Venturi, S. Subramoni, A. Sabag-Daigle, and B. M. Ahmer, "Methods to study solo/orphan quorum-sensing receptors," in *Quorum Sensing*. Berlin, Germany: Springer, 2018, pp. 145–159.

[38] S. Wellington and E. P. Greenberg, "Quorum sensing signal selectivity and the potential for interspecies cross talk," *mBio*, vol. 10, no. 2, 2019, Art. no. e00146–19.

[39] T. I. Yusufaly and J. Q. Boedicker, "Mapping quorum sensing onto neural networks to understand collective decision making in heterogeneous microbial communities," *Phys. Biol.*, vol. 14, no. 4, 2017, Art. no. 46002.

[40] J. J. Hopfield, "Neural networks and physical systems with emergent collective computational abilities," *Proc. Nat. Acad. Sci. USA*, vol. 79, no. 8, pp. 2554–2558, 1982.

[41] K. P. T. Silva and J. Q. Boedicker, "A neural network model predicts community-level signaling states in a diverse microbial community," *PLoS Comput. Biol.*, vol. 15, no. 6, 2019, Art. no. e1007166.

[42] G. L. Lozano et al., "Introducing THOR, A model microbiome for genetic dissection of community behavior," *mBio*, vol. 10, no. 2, 2019, Art. no. e02846–18, doi: 10.1128/mBio.02846-18.

[43] A. Hurley, M. G. Chevrette, N. Rosario-Meléndez, and J. Handelsman, "THOR's Hammer: The antibiotic koreenceine drives gene expression in a model microbial community," *mbio*, vol. 13, no. 3, 2022, Art. no. e02486–21.

[44] M. G. Chevrette et al., "Microbiome composition modulates secondary metabolism in a multispecies bacterial community," *Proc. Nat. Acad. Sci. USA*, vol. 119, no. 42, 2022, Art. no. e2212930119.