Stochastic effects in bacterial communication mediated by extracellular vesicles

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Quorum sensing (QS) allows bacterial cells to sense changes in local cell density and, hence, to regulate multicellular processes, including biofilm formation, regulation of virulence, and horizontal gene transfer. While, traditionally, QS was thought to involve the exchange of extracellular signal molecules free in solution, recent experiments have shown that for some bacterial systems a substantial fraction of signal molecules are packaged and delivered in extracellular vesicles. How the packaging of signal molecules in extracellular vesicles influences the ability of cells to communicate and coordinate multicellular behaviors remains largely unknown. We present here a stochastic reaction-diffusion model of QS that accounts for the exchange of both freely diffusing and vesicle-associated signal molecules. We find that the delivery of signal molecules via extracellular vesicles amplifies local fluctuations in the signal concentration, which can strongly affect the dynamics and spatial range of bacterial communication. For systems with multiple bacterial colonies, extracellular vesicles provide an alternate pathway for signal transport between colonies, and may be crucial for long-distance signal exchange in environments with strong degradation of free signal molecules.

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I. INTRODUCTION

Bacterial vesicles are small, spherical structures produced by a range of bacterial species in a variety of natural environments. Bacterial vesicles have been observed to range in size from 20 to 400 nm, and can form through budding of the bacterial membrane or as the result of cell lysis [1–4]. During their formation, bacterial vesicles can acquire biomolecular cargo. In particular, bacterial vesicles have been shown to contain biomolecules from the cytoplasm, such as proteins, DNA, and RNA [2,5–7]. Bacterial vesicles diffuse in the extracellular environment, and can be absorbed by both prokaryotic and eukaryotic cells, allowing delivery of biomolecular cargo to host cells and other bacteria [2].

Research over the past several decades has shed light on the variety of functions bacterial vesicles perform in communities of cells. In addition to transport of biomolecules, bacterial vesicles facilitate horizontal gene transfer, defend against viral attacks, remove misfolded proteins, and assist in the formation of biofilms [1,3,8,9]. Bacterial vesicles also play a role in infectious diseases by transporting virulence factors from infecting cells to the host cells [10–12]. More recently, it has been suggested that bacterial vesicles may have a significant influence on communication between bacteria, as bacterial vesicles are known to package signal molecules associated with quorum sensing (QS) [13–16].

During QS, bacteria release and detect signal molecules. At high enough signal-molecule concentrations, the binding of QS signal molecules to receptor proteins leads to changes in gene regulation, resulting in changes in cell phenotype [17]. Traditionally, it was thought that the exchange of QS signal molecules between cells was driven by diffusion of free signal molecules in the extracellular environment. However, recent work has demonstrated that within the extracellular environment, a substantial fraction of some types of QS signal molecules can be contained within bacterial vesicles. For instance, in cultures of *Paracoccus denitrificans*, it was found that as much as 34% of the total number of QS signal molecules may be contained within vesicles [13]. Similarly, Mashburn and Whitely [16] demonstrated that up to 86% of *Pseudomonas* quinolone signal (PQS) is associated with the vesicles produced by *Pseudomonas aeruginosa*. Moreover, it has been observed that bacterial vesicles carrying QS signal molecules could induce a QS response in populations of *P. denitrificans* and *Vibrio harveyi* [13,15], and that the removal of vesicles carrying PQS from cultures of *P. aeruginosa* halted QS-related behavior [16]. While it is now well established that bacteria produce vesicles to facilitate QS, the potential benefits and limitations of this mechanism for QS signal exchange remain unclear.

To analyze the role of bacterial vesicles in QS, we develop here a stochastic reaction-diffusion model of QS that allows for the exchange of both freely diffusing and vesicle-associated QS signal molecules between cells. On this basis, we compare QS in bacterial populations utilizing only freely diffusing QS signal molecules to QS in bacterial populations utilizing a combination of freely diffusing QS signal molecules and QS signal molecules packaged in vesicles. Bacterial vesicles may impact QS signal exchange dynamics in multiple ways, by slowing down the dispersal of signal molecules, by protecting signal molecules from degradation in the extracellular environment, and by amplifying local

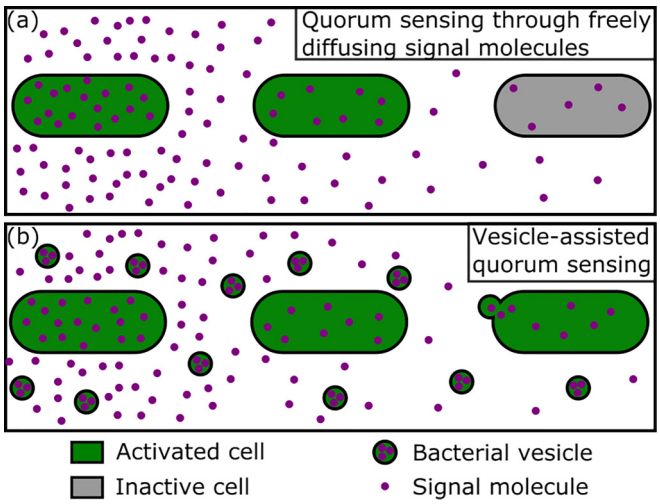

FIG. 1. Bacterial cells utilize two distinct pathways for the transport of QS signals. (a) In the classical model of QS, bacterial communication is mediated exclusively by freely diffusing signal molecules. (b) According to a newly emerging picture of cellular communication, cells utilize both freely diffusing signal molecules and signal molecules packaged in bacterial vesicles to facilitate QS.

TABLE I. Summary of reference parameter values employed for our simulations of QS. The values used here carry significant uncertainty but, as detailed in the footnotes, fall within the same order of magnitude as corresponding values used elsewhere in the literature.

Quantity	Units	Value
Signal diffusivity (D_f)	$\mu m^2/s$	80[a]
Signal decay rate (γ)	h^{-1}	0.01[b]
Fold change (f)	Dimensionless	6[c]
Activation threshold (c_{crit})	Molecules per grid spacing	100[d]
Basal production rate (r_b)	Molecules per hour per cell	2.15[e]
Vesicle diffusivity (D_v)	$\mu m^2/s$	2.5[f]

[a]Chosen with slight modification from the value in Ref. [20].
[b]Taken from the Supplementary Information in Ref. [22]. Matches the value in Ref. [25].
[c]Selected from the range 5–300-fold presented in Ref. [20], specifically to match Ref. [25].
[d]Chosen based on the range 10–70 nM reported in Ref. [26] and the value 450 nM reported in Ref. [19]. Matches value in Ref. [25].
[e]Chosen from the range provided in Ref. [19].
[f]Calculated from the Stokes-Einstein relation for a sphere of radius 100 nm diffusing in water.

fluctuations in QS signal-molecule concentration. We use our stochastic reaction-diffusion model of QS to compare these possible effects of bacterial vesicles on QS. We find that vesicle-induced amplification of local fluctuations in QS signal-molecule concentration has a dominant effect on signaling dynamics, decreasing the time and number of cells required to achieve activation of bacterial communities. Here and throughout this paper, we refer to the upregulation of QS-responsive genes in all cells in a bacterial community with the term "activation." To complement our simulations, we develop a simple analytic model that captures the increased fluctuations in QS signal concentration due to vesicles, and reproduces the basic trends in colony activation seen in our simulations (see Appendix A). Overall, we find here that bacterial vesicles can accelerate the QS response of bacterial populations. We also find that in systems of multiple bacterial colonies with a high rate of free signal degradation in the extracellular environment, vesicles can become essential for long-distance transport of QS signal molecules between bacterial colonies.

II. MODELING QUORUM SENSING

To analyze signal exchange between bacteria, we develop a stochastic reaction-diffusion model (see Fig. 1). Our model is based on a previous mean-field (deterministic) model of QS, which considered only freely diffusing signal molecules [18]. We modified this model to allow for stochastic effects in the diffusion of QS signal molecules and vesicles, as well as in the production and absorption of QS signal molecules and vesicles. To make our simulations more tractable from a computational perspective, we follow here Langebrake *et al.* [19] and take our system to be one dimensional (1D). Our model therefore does not capture quantitatively the diffusion of QS signal molecules in two-dimensional or three-dimensional bacterial colonies but, instead, is designed to allow investigation of qualitative differences between QS through freely diffusing signal molecules and vesicles. In our model, cells stochastically produce and take up both freely diffusing QS signal molecules and QS signal molecules packaged within vesicles. While the movement, division, and death of bacteria could be incorporated in the modeling framework described, here we take, for simplicity, the positions and numbers of cells to be fixed.

The free diffusion of QS signal molecules is modeled as a 1D random walk. At each time step, we allow signal molecules to randomly jump between neighboring points on a fixed grid defined by the diffusivity of free signal molecules, D_f, and the time step dt. Based on Ref. [20] we set $D_f = 80\ \mu m^2\ s^{-1}$ (see Table I). For the simulations described here we used the value $dt = 0.1563$ s. Together, these numbers set a grid spacing of 5 μm, the distance traveled by a free signal molecule over one time step in the simulations. This spacing is similar to the typical spacing between cells in bacterial biofilms [21]. We took neighboring cells in colonies to occupy neighboring points on this grid. Experiments suggest that freely diffusing signal molecules are subject to random decay with rate $\gamma = 0.01\ h^{-1}$ [22]. We assume that signal transport into and out of bacterial cells is rapid, and therefore take the concentration of free signal molecules inside a cell to be equal to the concentration of free signal molecules at the grid point occupied by that cell [23].

As a result of QS, bacterial cells do not produce signal molecules at a fixed rate but, rather, at a rate $r(c)$ that depends on the local concentration of free signal molecules, c. We measure here c in units of free signal molecules per grid point. For instance, in the paradigmatic QS system discovered in *Aliivibrio fischeri*, production of the LuxI enzyme depends directly on the concentration of QS signal molecules [17]. LuxI in turn catalyzes production of QS signal molecules [24],

leading to a positive feedback between the local concentration of signal molecules and the rate of signal production. This feedback approximately follows Michaelis-Menten kinetics, resulting in a sigmoidal dependence of r on c. Yusufaly *et al.* [25] demonstrated that a Heaviside step function, $\theta(c)$, provides a reasonable approximation of $r(c)$ when examining qualitative features of the activation of bacterial communities through QS. On this basis, we set

$$r(c) = r_b\{1 + f\theta[c(x,t) - c_{\text{crit}}]\}, \tag{1}$$

where r_b is the basal rate of signal production in each cell and c_{crit} is the critical concentration of free signal molecules for cells to transition to the activated state. Following Ref. [26] we set here $r_b = 2.15$ signal molecules per cell per hour and $c_{\text{crit}} = 100$ molecules per grid spacing (Table I). The parameter $f = 6$ in Eq. (1) models the fold change in the production rate of signal molecules in activated cells, as compared to inactivated cells [20]. In the following, we refer with "activation time" to the time point in our simulations at which all cells in a bacterial community have transitioned to the activated state.

In analogy to freely diffusing signal molecules, we model the movement of bacterial vesicles transporting QS signal molecules as a 1D random walk. The vesicles jump between locations on a fixed grid defined by the vesicle-diffusion coefficient D_v and the time step dt. The vesicle-diffusion coefficient can be (roughly) estimated from the Stokes-Einstein relation for the diffusion of a spherical particle with radius 100 nm in water, which results in $D_v \sim 2.5\ \mu\text{m}^2\ \text{s}^{-1}$, yielding a grid spacing $\sim 0.9\ \mu$m for vesicle diffusion. Similar values of D_v have also been measured in experiments on bacterial vesicles [27]. In Secs. III B and III D we adjust the vesicle-diffusion coefficient so as to match the diffusion coefficient of free signal molecules, resulting in a grid spacing of 5 μm. A change in the vesicle diffusivity could result from, for instance, a change in the vesicle radius. Experiments on *Prochlorococcus* have suggested vesicle production rates of the order of 2–5 vesicles per cell per generation [28]. As such, we take bacterial cells to generate, on average, one vesicle every 30 min, which is consistent with the doubling time of *Escherichia coli* under laboratory conditions [29].

When a new vesicle is produced by a bacterial cell we assume, for simplicity, that the vesicle is released from the center of the cell, and that all signal molecules at the grid location occupied by that cell are packaged into the newly generated vesicle. At the activation time, this results in each vesicle containing between approximately 20 and 100 signal molecules, with approximately 15% of all signal molecules in the system packaged in vesicles. In Appendix B we explore how our results change if not all signal molecules at the grid location occupied by a vesicle-producing cell are packaged into the newly generated vesicle. We assume that once the signal molecules are packaged into the vesicle, they are protected from decay until delivery to a cell through vesicle uptake [14]. Note that due to the 1D nature of our system, vesicles traveling across a colony cannot avoid contact with cells. *In vivo*, vesicle uptake appears to be a rare event. Newly produced vesicles are therefore unlikely to be immediately absorbed by the vesicle-producing cell or a nearest-neighbor cell. To avoid such artifacts in our 1D model, we assume that whenever a vesicle is within 1 μm of a cell center, uptake occurs with only a small probability 10^{-3}, which was chosen so that vesicles are likely to diffuse across the simulated bacterial colonies. During vesicle uptake, the vesicle releases signal molecules into the cell, adding the newly released signal molecules to the pool of free signal molecules at that grid location.

In our model, bacterial cells act as the source of all signal molecules, and as both a source and sink for vesicles. We represent bacterial colonies as cells occupying adjacent grid points. All simulated colonies were located near the center of the system and significantly smaller than the system size 10^5 μm used here, making boundary effects negligible for all the scenarios considered here. On the rare occasions that a signal molecule or vesicle did reach the system boundary, it was absorbed and removed from the system. We explored two distinct scenarios in our simulations. In the first, the system was populated with a single colony of cells. We compared the time for all cells in the colony to activate for different colony sizes with and without vesicles. Doing so allowed us to explore the influence of vesicles on intracolony signal exchange. In the second scenario, we considered two distinct bacterial colonies, and studied the activation dynamics as a function of colony separation. On this basis, we examined the influence of vesicles on intercolony QS.

In all our simulations we initialized the system, at time $t = 0$, with a fixed distribution of bacterial cells and no signal molecules anywhere in the system. The cells then begin to produce signal molecules according to Eq. (1) at a rate r_b. The signal molecules diffuse and decay according to the stochastic dynamics described above. To explore the effect of bacterial vesicles on QS, we separately considered situations in which bacteria do and do not produce vesicles. In systems containing vesicles, all cells can generate and absorb vesicles. The computer code for our simulations was written in MATLAB and run at the Center for Advanced Research Computing at the University of Southern California (USC). Each simulation contained as many as 10^7 random walkers (freely diffusing signal molecules or vesicles), and individual simulations took between 12 and 48 h on a single core. A typical dataset presented in this work includes ∼10 data points, with each data point produced by 10 simulation replicates. Access to a high-performance computing center allowed us to run simulation replicates in parallel.

III. SIMULATION RESULTS

In this section we use the stochastic reaction-diffusion model of QS described in Sec. II to examine the influence of vesicle-assisted transport of QS signal molecules on the activation dynamics of single bacterial colonies as well as interacting bacterial colonies. In particular, in Sec. III A we study how the activation time of a single colony depends on the colony size, and show that vesicle-assisted transport of QS signal molecules can substantially decrease the activation time. In Sec. III B we explore the physical mechanisms underlying the rapid activation of bacterial colonies through vesicle-assisted transport of QS signal molecules. We complement these simulations through a simple analytic model of colony activation (see Appendix A). Section III C examines the influence of bacterial vesicles on cellular activation dynamics in systems with two separate bacterial colonies.

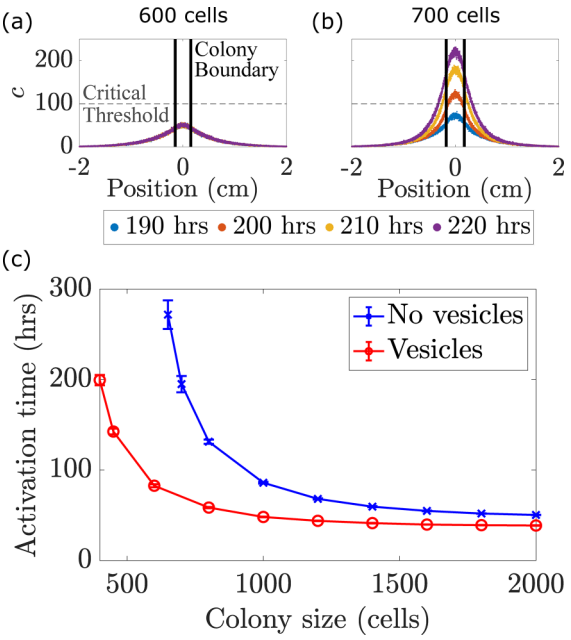

FIG. 2. Vesicle-assisted transport of QS signal molecules reduces the critical colony size required for colony activation and accelerates the activation dynamics. Time progression of the distribution of freely diffusing signal molecules for a system with no vesicle production with (a) 600 cells and (b) 700 cells. Vertical lines indicate colony boundaries and horizontal dashed lines indicate the critical concentration of signal molecules required for activation, $c_{\text{crit}} = 100$. The distributions shown are averages over ten simulation replicates, and the vertical axes are identical in both panels. (c) Time to complete colony activation vs colony size for simulations of single colonies with and without vesicle transport of signal molecules. Data points and error bars represent the average activation time and standard deviation from ten simulation replicates.

Finally, we consider in Sec. III D extracellular environments with pronounced degradation of free signal molecules, in which case vesicle-assisted transport of QS signal molecules becomes the primary means for the transport of QS signal molecules between distant colonies.

A. Signal transport with and without vesicles

In Fig. 2 we consider signal exchange and QS activation within a single colony of bacteria. Prior modeling of QS through freely diffusing signal molecules demonstrated that a critical colony size was needed to activate the QS response [30]. To examine the influence of vesicle-assisted transport of QS signal molecules on the critical colony size needed for activation, we ran simulations of the model described in Sec. II. We thereby compare QS activation for colonies in which all signal molecules are emitted as freely diffusing molecules to QS activation for colonies in which some fraction of the signal molecules is packaged into vesicles, using otherwise identical values of all model parameters.

We find that if cells produce no vesicles, bacterial colonies with 600 cells fail to activate QS at any point in our simulations [see Figs. 2(a) and 2(c)], while colonies with 700 cells locally yield signal concentrations above the critical threshold, and are hence able to activate [see Figs. 2(b) and 2(c)], similar to experimental results [30]. In contrast, allowing for vesicle-assisted transport of QS signal molecules, we find that colonies containing fewer than 500 cells are able to activate [Fig. 2(c)]. Interestingly, our simulations show that vesicles not only reduce the minimum colony size for activation of the QS response, but also enable colonies to activate at earlier times across all colony sizes considered here [Fig. 2(c)]. Thus, packaging signal molecules into vesicles appears to have two advantages: earlier activation and the potential to activate QS in smaller colonies. We note that some of the activation times in Fig. 2 are rather long compared to experimental results, >100 h for the smallest colonies as compared to around 10 h in experiments. Experiments are typically performed with much larger bacterial populations, far above the minimum colony size needed for self-activation, and, moreover, the total cell number may increase over time through cell division.

B. Mechanisms for improved signal transfer through vesicles

To understand why in Fig. 2(c) QS activation for bacterial colonies producing vesicles was more rapid and reduced the minimum colony size, we explored several potential benefits to packaging signal molecules into vesicles. First, vesicles diffuse more slowly than free signal molecules, reducing the rate at which released signal diffuses away from the colony. Second, vesicles protect signal molecules from decay through, for instance, interactions with enzymes in the extracellular environment, which overall leads to a more rapid accumulation of signal molecules in the system. Third, random vesicle uptake by cells results in a sharp increase in the intracellular signal concentration, as many signal molecules are delivered at once. To disentangle these effects of vesicle-assisted transport of QS signal molecules on colony activation, we modified our simulations so as to eliminate each of these effects one at a time, in order to isolate the dominant mechanism(s) underlying the results in Fig. 2(c).

Since bacterial vesicles diffuse more slowly than free signal molecules, vesicles effectively serve to retain QS signal molecules within the colony boundaries, which tends to bring the signal concentration closer to the critical activation concentration. For example, in Fig. 3(a) we show that after 25 h, the overall distribution of signal molecules is narrower in systems utilizing vesicles to transport signal molecules, resulting in a higher signal concentration within the colony. To test to what extent the slower diffusion of vesicles is responsible for the results in Fig. 2(c), we ran simulations in which the vesicle diffusion coefficient, D_v, was increased to match the diffusion coefficient of the free signal molecules, D_f. As shown in Fig. 3(b), setting $D_v = D_f$ only has a marginal effect on the colony activation times. These results indicate that the slow diffusion of vesicles does not have a strong effect on the QS activation dynamics of single colonies.

Next, we examined to what extent the slower degradation of QS signal molecules packaged in vesicles affects the colony activation time. Indeed, it has previously been suggested that since bacterial vesicles protect molecular cargo from degradation, bacterial vesicles may effectively allow for higher concentrations of biomolecules and, hence, more efficient transport [31]. To test whether protection of QS signal molecules from degradation through vesicles has a substantial

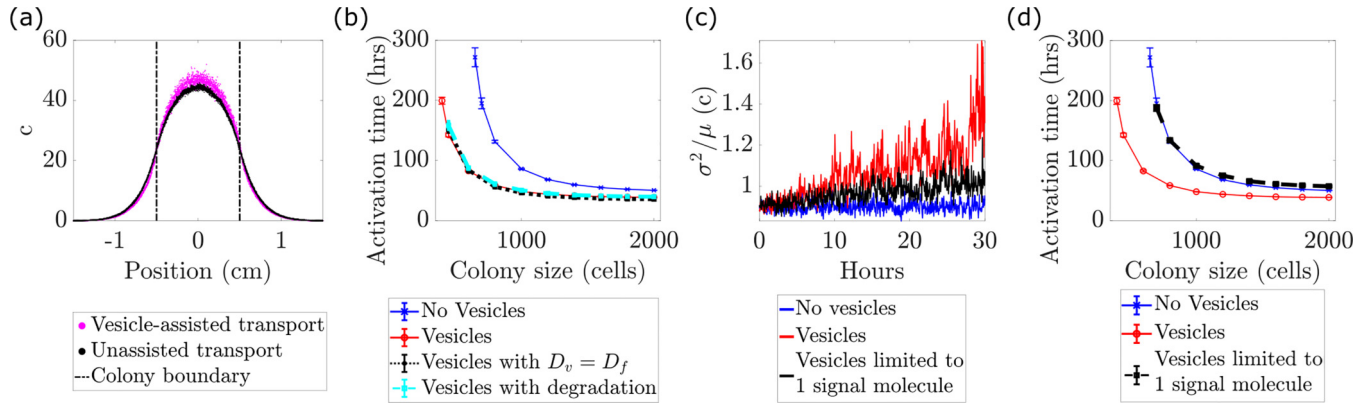

FIG. 3. Physical origins of improved signal transfer through vesicles. (a) Distribution of all QS signal molecules in a system of 2000 cells producing (magenta) or not producing (black) vesicles. Both distributions are shown at the same time point of 25 h. (b) Colony activation time vs colony size for simulations with $D_v = D_f$ (black dashed curve) and the same degradation rate γ for signal molecules packaged inside vesicles and freely diffusing signal molecules (cyan dashed curve), compared to the corresponding activation curves with and without vesicles in Fig. 2(c). (c) Fano factor for the signal concentration vs time for a cell at the center of a 2000 cell colony. The data shown here were obtained from a moving average with a 0.1-h time window. (d) Colony activation time vs colony size for cells producing vesicles with only one QS signal molecule per vesicle, compared to the corresponding activation curves with and without vesicles in Fig. 2(c) (see Appendix B for intermediate numbers of signal molecules packaged per vesicle). All results in this figure were obtained by averaging over ten simulation replicates with error bars representing standard deviations. Unless indicated otherwise, we used the same parameter values as in Fig. 2(c).

influence on colony activation dynamics, we ran simulations in which QS signal molecules degraded within vesicles at the same rate as freely diffusing signal molecules. As shown in Fig. 3(b), we thus found colony activation dynamics that were comparable to the activation dynamics obtained when vesicles protect QS signal molecules from degradation, suggesting that the decreased degradation of signal molecules within vesicles does not have a strong effect on the QS activation dynamics of single colonies.

Finally, we investigated to what extent the concentrated delivery of QS signal molecules through vesicles affects the colony activation dynamics. Since the number of QS signal molecules packaged in vesicles depends on the local concentration of signal molecules in cells, vesicles tend to contain fewer signal molecules at earlier times, and package an increasing number of signal molecules as the ambient concentration of free signal molecules in the system increases with time. In our simulations, this typically results in individual vesicles containing between 20 and 100 signal molecules when activation occurs. All of these signal molecules are delivered at once to cells when a vesicle uptake event occurs. In contrast, free signal molecules are absorbed gradually by cells, producing smaller fluctuations in the local concentration of QS signal molecules.

We illustrate the larger fluctuations induced by vesicles in Fig. 3(c) through the Fano factor—i.e., the ratio of the variance, σ^2, to the mean, μ—associated with the signal concentration at the colony center. Vesicles are indeed seen to increase fluctuations in the concentration of signal molecules. We tested to what extent the magnitude of these fluctuations affects the colony activation dynamics by modifying our simulations so that each vesicle was split into multiple vesicles, reducing the number of signal molecules per vesicle. For example, we adjusted our simulations so that instead of 1 vesicle with 20 signal molecules, 20 vesicles containing only 1 QS signal molecule each were produced. We thus ensured that the total fraction of signal molecules in vesicles remained unchanged. As shown in Fig. 3(d), reducing the number of signal molecules per vesicle increases the time needed for QS activation and, for one QS signal molecule per vesicle, results in colony activation dynamics similar to those found for colonies that do not produce any vesicles.

The results in Fig. 3(d) suggest that the ability of bacterial vesicles to deliver multiple QS signal molecules at once is primarily responsible for the more rapid QS activation, and the smaller minimum colony size required for activation, in Fig. 2(c). Note, in particular, that if a colony that is not producing any vesicles is slightly too small to activate QS, it will have a steady-state concentration of signal molecules just below the critical activation threshold. Fluctuations in the concentration of signal molecules can bring individual cells above the critical activation threshold, but for freely diffusing signal molecules these fluctuations are small. Vesicle-assisted signal transport amplifies fluctuations in the local concentration of signal molecules, which can potentially drive the local concentration of signal molecules above the critical activation concentration, thus decreasing the activation time and allowing smaller colonies to activate QS. In Appendix A we further explore this physical picture through a simple analytic (Poisson) model designed to isolate the effect of local fluctuations in the signal concentration on colony activation. We show in Appendix A that our analytic model is able to reproduce the basic trends in colony activation time found in our simulations in Figs. 2 and 3.

C. Signal transfer between colonies

The results in Secs. III A and III B show that vesicles can substantially affect signal transfer between cells within the same colony. But, how do vesicles affect communication between spatially segregated bacterial communities? It was demonstrated previously that for a system with multiple bacte-

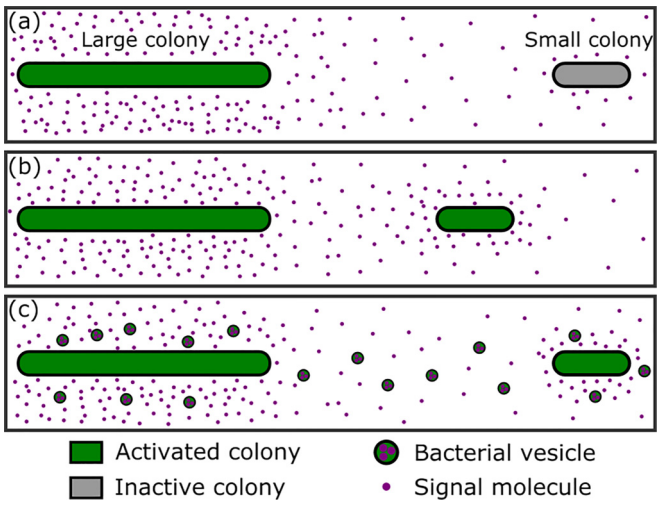

FIG. 4. Large colonies can activate smaller colonies that are otherwise unable to activate. (a) For large enough colony separations, the larger colony does not activate the smaller colony over a given timescale, while (b) over the same timescale activation of the smaller colony by the larger colony may be achieved at smaller separations. (c) Bacterial vesicle production can result in more rapid activation of the smaller colony by the larger colony, and yield intercolony activation over larger colony separations.

rial colonies distributed in space, QS signal exchange between colonies can enable activation of cells within small colonies that otherwise would not be able to activate [25,30]. It is currently unknown what effect vesicle-assisted signal transport might have on intercolony QS. To explore this question, we considered a system with two spatially separated colonies (see Fig. 4). One colony was large enough to self-activate through QS. The other colony was smaller so that by itself it was unable to activate over the timescales considered in our simulations. Starting with no QS signal molecules in the system, we studied the time at which all cells within the two colonies became activated [see Fig. 5(a)]. We find that vesicle-assisted signal transport yields more rapid activation of the two-colony system for all the colony separations considered in Fig. 5(a).

The more rapid colony activation for vesicle-producing bacteria in Fig. 5(a) could arise from more efficient intercolony transport of signal molecules through vesicles, or from the faster activation of individual colonies found in Secs. III A and III B for vesicle-producing bacteria. For the scenarios considered in Fig. 5(a), we find a very small rate of vesicle transfer between colonies [see Fig. 5(b)], indicating that intercolony vesicle transfer does not substantially affect colony activation in Fig. 5(a). Indeed, repeating our simulations while only allowing vesicles to be taken up by cells in their colony of origin, we find no noticeable effect on the activation time of the smaller colony [Fig. 5(a)]. This implies that for the model parameters considered here, the transfer of vesicles between colonies does not play an important role in the activation of a small colony by a neighboring larger colony. Instead, we attribute the more rapid activation of QS for vesicle-producing bacteria in Fig. 5(a) to the faster activation of the larger colony through vesicles produced by that colony.

The results in Fig. 5 suggest that the ability of vesicles to directly influence intercolony QS is mainly limited by the slow diffusion of bacterial vesicles. The diffusion coefficient of bacterial vesicles could be increased, for instance, by decreasing the vesicle size. Upon repeating our simulations with a (strongly) increased vesicle-diffusion coefficient, we find substantial vesicle transfer for a wide range of intercolony separations [Fig. 5(b)]. With such rapid diffusion of bacterial vesicles, vesicle-assisted signal transport between colonies can further lower the activation time for intercolony QS [see Fig. 5(c)]. However, overall, we find that for the reference

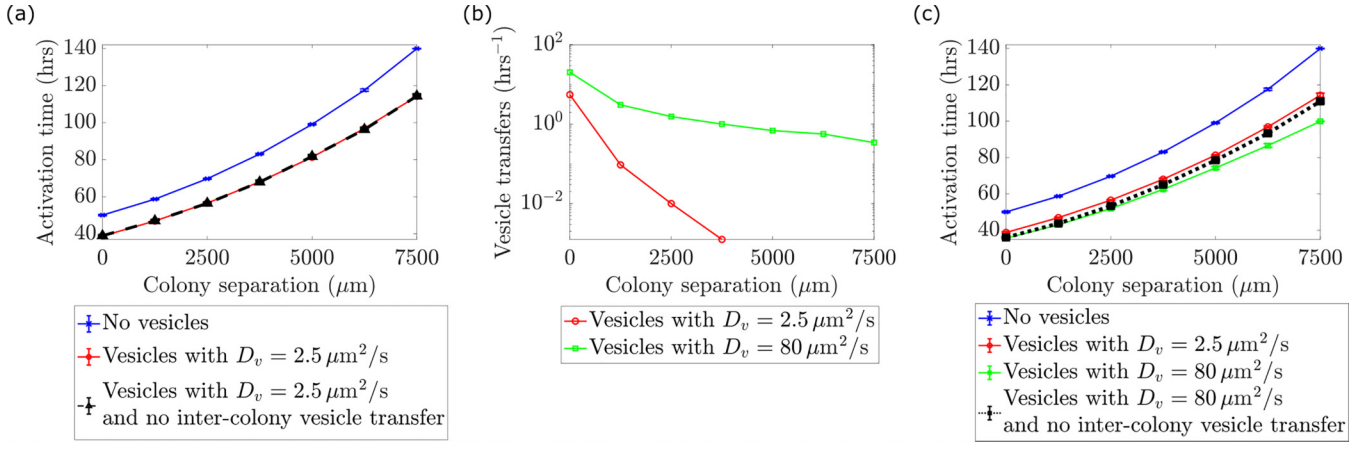

FIG. 5. Vesicle-assisted signal transport in a two-colony system. (a) Time to complete activation of a 100-cell colony vs edge-to-edge separation from a neighboring 2000-cell colony, for bacteria producing no vesicles (blue curve), bacteria producing vesicles (red curve), and bacteria producing vesicles with no intercolony vesicle transfer (black dashed curve). (b) Average rate of vesicle transfer from the larger colony to the smaller colony from $t = 0$ to complete activation of the smaller colony vs colony separation for the reference value of the vesicle-diffusion coefficient used here, $D_v = 2.5\ \mu\text{m}^2/\text{s}$ (red curve), and an increased vesicle-diffusion coefficient, $D_v = D_f = 80\ \mu\text{m}^2/\text{s}$ (green curve). (c) Time to complete activation of the smaller vs colony separation as in panel (a), with the increased diffusion coefficient $D_v = D_f = 80\ \mu\text{m}^2/\text{s}$ (green curve) and $D_v = D_f = 80\ \mu\text{m}^2/\text{s}$ but no intercolony vesicle transfer (black dashed curve). We used the same colony sizes for all panels. The data points show averages and the error bars indicate the corresponding standard deviations from ten simulation replicates each.

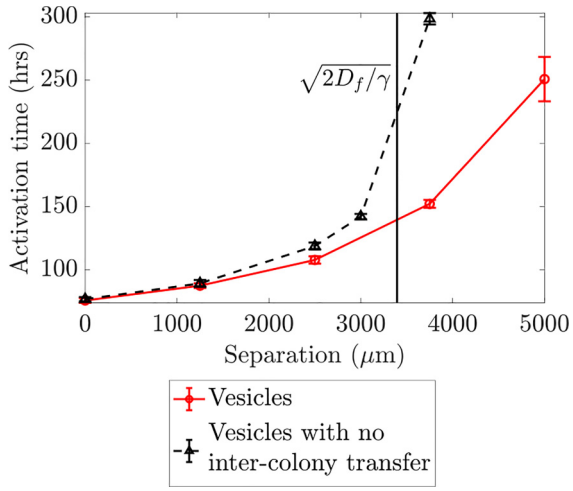

FIG. 6. Intercolony vesicle exchange can strongly affect colony activation for fast enough degradation of free signal molecules and rapid vesicle diffusion. Time to complete activation of a 100-cell colony vs edge-to-edge separation from a neighboring 2000-cell colony. Free signal decay is increased here such that $\gamma = 0.05\ \mathrm{h}^{-1}$ and the vesicle-diffusion coefficient is increased such that $D_v = D_f = 80\ \mu\mathrm{m}^2\ \mathrm{s}^{-1}$, resulting in the characteristic length scale $\sqrt{2D_f/\gamma} \approx 3400\ \mu\mathrm{m}$. Similarly as in Fig. 5, the curves shown compare scenarios in which vesicles can (red curve) and cannot (black curve) contribute to signal exchange between colonies.

parameter values used here, local reabsorption of vesicles within a colony has a much larger effect on intercolony QS than vesicle exchange between distant colonies.

D. Signal transfer under rapid signal degradation

In Sec. III C we concluded that for the reference parameter values used here, intercolony exchange of bacterial vesicles does not play an important role in the activation of a smaller bacterial colony by a larger colony. This conclusion is, however, expected to depend on the particular set of simulation parameter values used. For example, in an environment with high degradation rates of free signal molecules, the protection of signal molecules from degradation afforded by vesicles might give bacterial vesicles a more prominent role in intercolony communication. Furthermore, the foregoing results also suggest that if vesicles diffuse more quickly, which could come about through a decrease in the size of vesicles produced by bacteria, vesicles may contribute more substantially to intercolony communication.

To test the potential role of vesicles in long-distance signal transport, we simulated vesicle exchange between two colonies with faster degradation of free signal molecules in the extracellular environment and more rapid diffusion of vesicles (see Fig. 6). In particular, we proceeded as in Fig. 5, but with the free signal degradation rate $\gamma = 0.05\ \mathrm{h}^{-1}$, rather than $\gamma = 0.01\ \mathrm{h}^{-1}$, and the vesicle diffusion coefficient $D_v = D_f = 80\ \mu\mathrm{m}^2\ \mathrm{s}^{-1}$. These parameter values yield the characteristic length scale $\sqrt{2D_f/\gamma} \approx 3400\ \mu\mathrm{m}$ over which diffusion of free signal molecules is expected to begin to compete with degradation of free signal molecules [18], which roughly determines the (maximum) scale of colony separations over which free signal molecules may contribute to intercolony QS. Similarly as in Fig. 5(a), we compare in Fig. 6 scenarios in which the transfer of vesicles between colonies is prohibited to scenarios in which it is allowed, so as to isolate the influence of vesicle transfer on the activation of the smaller colony. We find that if vesicle exchange between colonies is prohibited, the activation time of the smaller colony rapidly increases beyond the characteristic length scale $\sqrt{2D_f/\gamma}$, suggesting a potentially important role of vesicles in long-distance bacterial communication in environments with fast degradation of free signal molecules and rapid vesicle diffusion.

IV. DISCUSSION AND CONCLUSION

Several recent studies have shown that bacterial QS signal molecules can be transported by extracellular vesicles [2,13–15,32,33]. Such vesicles have been found to activate QS-responsive gene expression, but the precise role of vesicle-mediated signal exchange between bacteria has not been explored. Vesicles containing QS signal molecules have been reported in a variety of bacterial systems, including cells that utilize acyl homoserine lactone of varying tail lengths [13,15,33] and PQS [16,32]. In some contexts, a large fraction of the signal molecules was found to be packaged in vesicles [13,16]. Our aim here was to develop a theoretical model of vesicle-mediated QS, to understand how vesicles may influence QS signal exchange within bacterial communities.

Based on a stochastic model of QS we have shown that bacterial colonies using vesicles to transport QS signal molecules are able to activate their QS response more quickly, and with smaller colony sizes, than colonies utilizing only freely diffusing signal molecules. There are many potential advantages to packaging signal molecules within vesicles, including protection from degradation in the environment and reducing the rate of diffusion of signal molecules away from the bacterial colony. Based on parameter values for the production, degradation, and diffusion of bacterial vesicles and free signal molecules estimated from previous studies, we find that an important aspect of signal exchange through vesicles is their ability to deliver many signal molecules at once. In the simulations presented here, vesicles generally contained 100 or fewer signal molecules, but it has been reported that, in general, vesicles can be loaded with up to 10^5 signal molecules [13]. Given that QS activation threshold concentrations for many QS signals are in the nM range—i.e., approximately 1 to 500 molecules per cell [19]—uptake of even one vesicle can initialize the changes in gene expression associated with QS activation. Vesicles in our simulations typically contained fewer QS signal molecules than needed for activation of a single cell. However, vesicles still greatly increased the local fluctuations in the signal concentration, leading to earlier activation of QS with smaller colony sizes than found with only freely diffusing signal molecules. These results can be rationalized through a simple analytic model of colony activation (see Appendix A). Because QS activation triggers a highly nonlinear positive feedback in the production of QS signal molecules, activation of even just a few cells within a colony can, ultimately, yield activation of the entire population of a bacterial colony.

We found here that increased fluctuations in the intracellular QS signal concentration due to vesicle uptake can produce

more rapid QS activation in spatially separated colonies. At distance scales beyond the characteristic length scale defined by the diffusion and degradation of free signal molecules, the protection from signal degradation provided by vesicles may become increasingly important in intercolony QS. In particular, we found that in environments with rapid degradation of free signal molecules, the transfer of vesicles between colonies can become essential for intercolony activation at large enough colony separations.

The stochastic model of QS described here was motivated by prior experiments on bacterial signal exchange. But, we made a number of simplifying assumptions that need to be carefully tested in future work. In particular, we assumed that when a cell generates a vesicle, 100% of the signal molecules currently residing in that cell are packaged in the vesicle. For the parameter values employed here, this resulted in $\sim$15% of all signal molecules in the system residing in vesicles at any given time. In reality, cells may not package all currently available signal molecules in vesicles. Decreasing this packaging fraction, we find that the extent to which vesicles affect signal exchange is diminished, but that our basic conclusions regarding the influence of bacterial vesicles on QS are left unchanged. We also assumed here that once vesicles are formed, they do not lose or gain signal molecules over time. The extent to which the number of signal molecules present in vesicles can be regarded as constant in time is likely to depend on the chemical properties of the signal molecules under consideration, how easily the signal molecules cross the vesicle membrane, and the signal-molecule concentration in the local environment. Substantial exchange of signal molecules between vesicles and their environment may limit the ability of vesicles to deliver signal molecules over long distances between bacterial colonies, as the gradient of free signal molecules would likely taper off between colonies, which could in turn lead to significant depletion of signal molecules in vesicles over time.

Our stochastic model of QS assumes a given, fixed vesicle-diffusion coefficient for each scenario considered. In reality, bacterial vesicles are produced in a range of sizes even in communities composed of only one bacterial species, which may yield heterogeneity in the diffusive behavior of vesicles. Such heterogeneity in the vesicle-diffusion coefficient and, hence, vesicle-based signal exchange may confer additional advantages to vesicle-based bacterial communication. Furthermore, if bacteria can control the size of the vesicles employed for signal exchange, they may be able to tune vesicle-based signal exchange to a given environment. In particular, our results suggest that in environments with rapid degradation of free signal molecules, smaller vesicles, which diffuse more quickly, may be advantageous for intercolony communication.

We note that the 1D nature of our simulations means that our model predictions are qualitative rather than quantitative. But, we expect the basic mechanisms for signal transport through vesicles explored here to apply to natural systems. Simulations of our model in two or three dimensions would be more realistic, but in the current computational implementation of our model the computational demands for biologically realistic system sizes would be prohibitive. These challenges could potentially be addressed through an improved computational design of our simulations taking full advantage of parallel programming optimized for large computing clusters. In this context, it would be particularly interesting to consider two-dimensional systems in which the added spatial dimension shows reflecting boundaries and is significantly restricted, a scenario that would correspond to bacterial colonies in narrow channels. In the 1D system we focused on here, we took the system boundaries to be absorbing and a great distance away from the colonies of interest, making their influence on the colony activation dynamics negligible. Allowing QS signal molecules to diffuse away from colonies in an additional dimension would effectively reduce the QS signal concentration inside each cell, with the amount by which the signal concentration is reduced being controlled by the channel width, while reflecting channel boundaries would prevent any loss of molecules in this direction. In the limit of an infinitely narrow channel, the distribution of QS signal molecules in the channel cross section can be visualized as a Dirac delta function. As the channel cross section is increased, the distribution of QS signal molecules in the channel cross section is expected to spread out and to eventually take the (approximate) form of a Gaussian once the channel boundaries are sufficiently far from the colony. Quantitatively, such simulations are expected to give results distinct from those presented here. Even in such two-dimensional (or three-dimensional) systems vesicles would, however, retain their ability to deliver concentrated packets of QS signal molecules, thus preserving their ability to amplify fluctuations in the local signal concentration and drive earlier QS activation.

Experiments suggest that not all types of bacterial signals are packaged within vesicles. For example, *P. aeruginosa* produces two kinds of homoserine lactone signal molecules in addition to PQS, but preferentially packages PQS in vesicles [16]. This suggests that there are unique advantages and disadvantages to utilizing vesicles for signal transport. Our results indicate that signal exchange through vesicles reduces the time needed to activate QS and enables smaller colonies to self-activate, but such earlier activation of QS may not always be advantageous. In general, QS systems are thought to have evolved to delay the onset of energetically costly phenotypes [34,35], and the benefits of QS activation increase with cellular density [36]. Thus, early activation of bacterial populations can be disadvantageous if the population size is too small. In particular, it may be disadvantageous for cells whose QS-regulated behaviors are particularly costly to package signal molecules into vesicles. Furthermore, given the increased fluctuations in signal concentration due to vesicle exchange, signaling networks utilizing vesicles may be less precise, especially for populations near their critical size for QS activation. A combination of free signal molecules and signal molecules packaged in vesicles may allow for a bet-hedging strategy, such that only a fraction of isolated populations activates QS.

Vesicles may have the ability to carry signal molecules over extended periods of time, which could provide an important advantage for communication between populations of cells that are far apart from each other. Molecular gradients generated by diffusion are poor at molecular exchange over distances of several millimeters, especially when coupled to background degradation in the environment. Vesicles overcome this limitation both by protecting signal molecules from

the environment and by delivering concentrated packets of signal molecules to distant cells. In this context, it is interesting to note that as pointed out above, vesicles may package enough signal molecules to activate an individual cell. This suggests that the transfer of even only one vesicle to a distant population of cells could lead to QS activation of that population of cells, via long-distance signal exchange. Importantly, the local concentration of signal molecules within vesicles can remain above the critical concentration for QS activation irrespective of transport conditions. Even under potentially unfavorable conditions, such as in the presence of convective flow, vesicles could thus allow coordination of QS between colonies of cells.

In particular, convective fluid flows have the potential to wash away QS signal molecules and, indeed, QS activation has been found to occur later and to require a greater mass of cells under conditions of strong fluid flow [37]. In future work it would be interesting to consider the influence of fluid flow on vesicle-based activation in and between colonies, as the difference in diffusivity between vesicles and freely diffusing molecules can potentially alter the properties of signal transport within a fluid flow. Importantly, the Schmidt number (Sc), a ratio of viscosity to diffusivity within the fluid, is expected to be greater for vesicles than for freely diffusing signal molecules. This implies that under identical fluid-flow conditions, vesicles and freely diffusing molecules could have distinct transport properties. Notably, in very large populations of cells, where the spatial scale of the colony extends beyond the effective scale of diffusive transport, fluid flow could be a significant factor in populationwide QS activation, with the greater Sc associated with vesicles making vesicles more effective at the targeted delivery of QS signal molecules.

In the stochastic reaction-diffusion model of QS considered here we focused on homogeneous colonies; however, a great deal of QS occurs within spatially inhomogeneous biofilms. The extracellular matrix present in biofilms segregates the constituent cells, and limits the ability of QS signal molecules to move from one segregated cell cluster to another. Under these conditions, vesicles carrying concentrated packets of QS signal molecules could significantly ease the difficulty of delivering a sufficient amount of QS signal molecules to achieve activation, provided that at least some vesicles are able to move in between the segregated biofilm compartments. In particular, biofilms can contain interior channels ~ 100 μm in diameter, in which fluid flows between segregated volumes of the biofilm matrix [38]. Assuming that vesicles are able to travel through biofilm channels, such channels could effectively become transport highways shuttling concentrated loads of QS signal molecules (as well as other molecules) between segregated regions of the biofilm matrix.

The simulations described here focused on QS, but our modeling framework is more broadly applicable to the transport of molecular cargo through vesicles. The highly concentrated delivery of molecular cargo during vesicle uptake events may be beneficial for cargo transport in a variety of contexts. For instance, *P. aeruginosa* uses vesicles to simultaneously deliver multiple virulence factors to host cells, coordinating and enhancing infection [10]. *P. aeruginosa* vesicles also deliver concentrated packages of DNA, allowing horizontal transfer of multiple genes in parallel, which in turn leads to more efficient infectious activity and community behavior [5]. Furthermore, vesicles can protect extracellular RNA from RNA-degrading RNases [6], and allow extracellular RNA to persist in environments where the timescale of RNA degradation is short enough to prevent effective RNA transport through free diffusion. We have found here, in the context of QS, that vesicle-based molecular transport has unique advantages over standard molecular transport through free diffusion. It would be interesting to explore in future work the role of vesicles in concentrating and ferrying varied types of molecular cargo, and how this transport pathway may provide unique advantages in diverse environments and bacterial communities.

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APPENDIX A

Section III B of the main text suggests that the primary property of vesicles responsible for earlier colony activation times is the ability of vesicles to deliver many QS signal molecules at once. Figure 3(c) demonstrates that the Fano factor, a measurement of fluctuations, is larger and increasing in time for simulations with some fraction of signal transported by vesicles. To provide further insight into these results, we consider in this appendix a highly simplified analytic model of colony activation. In this analytic model, we randomly distribute packets of signal molecules (free QS signal molecules or vesicles) among equally spaced sites in a homogeneous system, with each packet containing α signal molecules. We take the boundaries of the system to be periodic, thus ignoring the effects of signal molecules leaving the system. In this sense, our simplified model applies most directly to the bulk behavior of colonies with large numbers of cells. We will discuss below how boundary effects arising from a finite colony size can be incorporated into this framework. Under these assumptions, the mean number of packets at each grid location is given by

$$\mu_v = \sum_n np(n), \tag{A1}$$

where n is the number of packets at a given grid location and $p(n)$ denotes the probability of having n packets at this grid location. Similarly, the variance in the number of packets per lattice site is given by

$$\sigma_v^2 = \sum_n n^2\, p(n) - \mu_v^2. \tag{A2}$$

The mean number of signal molecules per grid location can therefore be written as

$$\mu_m = \sum_n \alpha np(n) = \alpha \mu_v, \tag{A3}$$

with variance

$$\sigma_m^2 = \sum_n (\alpha\, n)^2\; p(n) - \mu_m^2 = \alpha^2\, \sigma_v^2. \tag{A4}$$

Using Eqs. (A3) and (A4), we can write the Fano factor of the signal molecule concentration, F_m, in terms of the packet size α and of the Fano factor associated with packets of signal molecules, F_v,

$$F_m = \frac{\sigma_m^2}{\mu_m} = \frac{\alpha^2 \sigma_v^2}{\alpha \mu_v} = \alpha F_v. \tag{A5}$$

Prior to colony activation, we expect the packets of signal molecules to be distributed independently of each other in our system. We therefore take $p(n)$ to be a Poisson distribution. We then have $\mu_v = \sigma_v^2$, $F_v = 1$, and $F_m = \alpha$. Thus, when signal molecules are transported in packets of size α, the Fano factor of the number of molecules at a given location is equal to the packet size α. For freely diffusing signal molecules, $\alpha = 1$. For vesicles, the packet size increases with the number of signal molecules produced in the system. This means that in the case of freely diffusing signal molecules, the Fano factor is expected to be approximately constant with time, while for signal molecules packaged in vesicles, the Fano factor is expected to increase with time, as more and more signal molecules are packaged in vesicles. These properties of the simple analytic model considered here agree with the simulation results in Fig. 3(c).

To estimate how vesicles affect fluctuations in a system with both freely diffusing signal molecules and vesicles, we allow for two distinct types of packets with distinct values of α, $\alpha_1 = 1$ (freely diffusing signal molecules) and $\alpha_2 > 1$ (vesicles). We approximate the mean number of signal molecules per grid location through

$$\mu_{\text{sig}} \approx r_b t = \alpha_1 \mu_{\text{free}} + \alpha_2 \mu_{\text{ves}}, \tag{A6}$$

where, as in the main text, r_b denotes the basal rate of signal production. In Eq. (A6) we ignore signal decay and take vesicle uptake to occur instantaneously. We have the mean free signal and vesicle concentrations $\mu_{\text{free}} = (1-A) r_b t$ and $\mu_{\text{ves}} = A r_b t/\alpha_2$ in Eq. (A6), with A denoting the fraction of all signal molecules in the system being transported by vesicles. We thus have the combined variance:

$$\sigma_{\text{sig}}^2 \approx (1 - A) r_b t + \alpha_2 A r_b t. \tag{A7}$$

Based on Eqs. (A6) and (A7) we can numerically estimate the effect increased fluctuations in the signal-molecule concentration due to vesicles have on colony activation. To this end, we consider the temporal evolution of a fluctuation envelope one standard deviation in width and centered about the mean signal concentration, c_{env}. Note that to ensure $\mu_{\text{sig}} \approx r_b t$ in Eq. (A6), we must have $\alpha_2 = A r_b t/\mu_{\text{ves}}$. Equations (A6) and (A7) then yield

$$c_{\text{env}} = \mu_{\text{sig}} + \sigma_{\text{sig}} = r_b t + \sqrt{(1 - A) r_b t + \frac{(A r_b t)^2}{\mu_{\text{ves}}}}. \tag{A8}$$

We take colony activation to occur when c_{env} equals the colony activation threshold, $c_{\text{env}} = c_{\text{crit}}$. For given values of r_b, μ_{ves}, and A, we can then use Eq. (A8) to estimate the colony activation time with and without vesicles. For simulations in the main text with a single, vesicle-producing colony, we have $A \approx 0.15$ and $\mu_{\text{ves}} \approx 0.25$ per lattice site, while $A = 0$ for colonies that do not produce any vesicles. Furthermore, we set $r_b = 2.15$ molecules per hour per cell in our simulations (see Table I). With these parameter values, Eq. (A8) yields the approximate colony activation times 42 and 35 h without and with vesicles, respectively. For colonies with approximately 2000 or more cells, these estimated colony activation times match reasonably well the corresponding activation times 50 and 39 h obtained in our simulations without and with vesicles (see Figs. 2 and 3). We note that the aforementioned estimates obtained from Eq. (A8) only account for fluctuation envelopes 1 standard deviation in width. Considering fluctuation envelopes 2 standard deviations in width, the estimated activation time would be 28.3 h with vesicles, and 38.1 h without vesicles. Similarly, considering fluctuation envelopes 3 standard deviations in width, the estimated activation time would be 23.5 h with vesicles, and 34.5 h without vesicles.

For small enough colony sizes, the activation times in Figs. 2 and 3 are substantially greater than the above estimates obtained from Eq. (A8). This can be understood by noting that the simplified model considered so far applies most accurately to large systems of cells with "bulk" conditions. In small enough colonies, the timescale for freely diffusing signal molecules to diffuse out of the colony becomes short enough that the approximation $\mu_{\text{sig}} \approx r_b t$ underlying Eqs. (A6)–(A8) is no longer valid. In this case, we instead have a reduced mean signal concentration in the colony, $\mu_{\text{sig}} \approx \beta r_b t$, where $\beta < 1$ represents the reduction in QS signal-molecule concentration. In this way, c_{env} can be modified to account for smaller colonies, and will predict increasingly long activation times as $\beta \to 0$. To avoid directly calculating β, we repeated our simulations without vesicles and without allowing cells to activate, and tracked the concentration of signal molecules at the center of the colony as a function of time. We assumed μ_{sig} to be equal to this center concentration, and thus used Eq. (A8) to estimate the colony activation time as a function of colony size for systems with and without vesicles. In Fig. 7 we compare the resulting estimated colony activation times to the corresponding activation times obtained in the stochastic simulations described in the main text. We find that our simple estimates provide reasonably good agreement with our simulation results if we consider a fluctuation envelope corresponding to $\mu_{\text{sig}} + 3\sigma_{\text{sig}}$, rather than the fluctuation envelope $\mu_{\text{sig}} + \sigma_{\text{sig}}$ in Eq. (A8). Figure 7 shows that our simple model captures the basic trends in activation time as the colony size is decreased in our stochastic simulations. This again indicates that increased fluctuations in the QS signal-molecule concentration due to the concentrated delivery of QS signal molecules through vesicles explains the decreased activation times for vesicle-producing colonies found in our stochastic simulations.

The estimated activation curves in Fig. 7 terminate for small enough colony sizes because, for such colony sizes, c_{env} failed to reach the critical signal concentration during our simulations. If we were to consider even larger and less probable fluctuations beyond $\mu_{\text{sig}} + 3\sigma_{\text{sig}}$, it would become possible to estimate the corresponding activation times for these smaller colony sizes. Interestingly, this suggests that

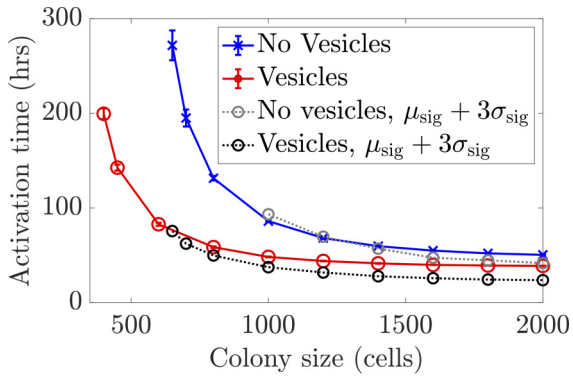

FIG. 7. Estimated colony activation time vs colony size without (gray) and with vesicles (black) as in Eq. (A8) but with a fluctuation envelope corresponding to $\mu_{sig} + 3\sigma_{sig}$ and QS signal-molecule concentrations estimated from simulations without vesicles and without allowing cells to activate. The blue and red curves are identical to the corresponding results in Figs. 2 and 3 and reproduced here for ease of comparison.

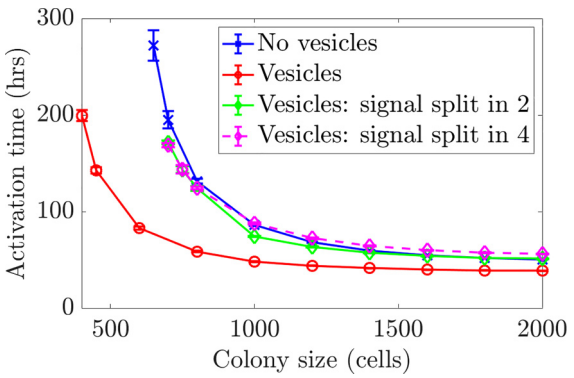

FIG. 9. Colony activation time vs. colony size obtained with no vesicles, with the algorithm for vesicle generation employed in the main text, and with the signal molecules being split evenly between two or four vesicles at vesicle-generation events. Splitting signal molecules among multiple vesicles at vesicle-generation events reduces the number of signal molecules per vesicle, and increases the activation time. All data points and error bars were calculated from ten simulation replicates.

small colonies close to the minimum colony size required for activation rely on relatively improbable fluctuations for their activation.

APPENDIX B

Our stochastic model of QS assumes that, when a vesicle is generated by a cell, some fraction of the signal molecules in that cell is packaged into the vesicle. Throughout the main text we made the simplifying assumption that this fraction was equal to 1, $f_{pack} = 1$. In reality, f_{pack} is likely to be less than 1 although, to our knowledge, no direct measurements of f_{pack} are currently available. We find that vesicles can remain advantageous for QS even if cells do not package all their currently available signal molecules into vesicles. For instance, Fig. 8 shows that with $f_{pack} = 0.5$, the colony activation times are shifted closer to the activation times associated with systems lacking vesicles. However, the primary beneficial effects

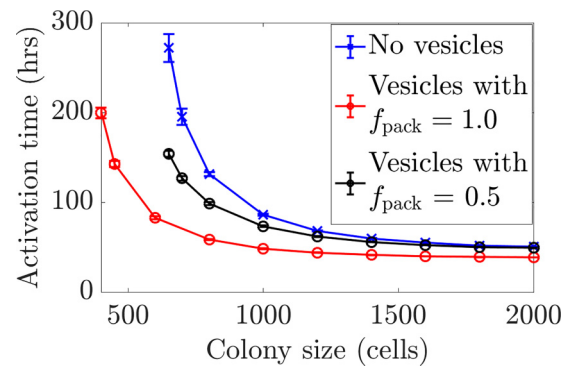

FIG. 8. Colony activation time vs. colony size obtained with no vesicles, vesicles with $f_{pack} = 1.0$, and vesicles with $f_{pack} = 0.5$. Reducing the fraction of signal molecules packaged into vesicles weakens the overall effect of vesicles on the colony activation dynamics. The data points and error bars were calculated from ten simulation replicates.

of vesicles on QS—i.e., earlier colony activation and reduced minimum colony size for QS—remain present.

Figure 3 of the main text shows that the number of signal molecules per vesicle can have a strong effect on the colony activation time. To further investigate how the number of signal molecules in vesicles affects the colony activation dynamics, we examine in Fig. 9 systems with $f_{pack} = 1.0$ in which vesicle production was modified so that not all signal molecules were packaged into a single vesicle but, rather, the signal molecules were evenly split among simultaneously generated vesicles [see also Fig. 3(d)]. In cases where the number of signal molecules packaged into vesicles could not be divided evenly among vesicles, the remaining signal molecules were thereby packaged into an additional vesicle. We find that splitting up signal molecules among, for instance, two or four vesicles can have a pronounced effect on the colony activation time, confirming the importance of the number of signal molecules per vesicle for the colony activation time. It is instructive to explicitly examine the relation between the colony activation time and the average number of signal molecules per vesicle (see Fig. 10). As expected, we find that the colony activation time decreases with increasing average number of signal molecules per vesicle.

Furthermore, it is instructive to examine in more detail the activation times obtained in Fig. 8 for $f_{pack} = 0.5$ and in Fig. 9 with the signal molecules being divided equally between two vesicles, the former showing slightly more rapid colony activation dynamics despite the total fraction of signal molecules in vesicles being smaller. If we consider a particular cell containing N signal molecules, either method of generating vesicles results, on average, in $N/2$ signal molecules in each vesicle. However, for $f_{pack} = 0.5$, $N/2$ signal molecules remain in the cell generating the vesicle and are thus available for future vesicle generation events, increasing the number of signal molecules in subsequently generated vesicles (Fig. 10), which may explain the somewhat more rapid colony activation found with $f_{pack} = 0.5$ in Figs. 8 –10.

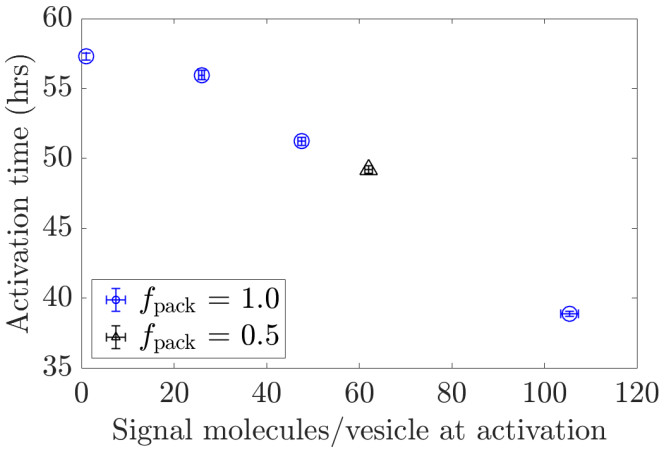

FIG. 10. Activation time in a 2000-cell colony vs average number of signal molecules per vesicle at the time of colony activation. The blue data points correspond to scenarios with one molecule per vesicle, to scenarios with the signal molecules being split evenly among four vesicles at vesicle generation events, to scenarios with the signal molecules being split evenly among two vesicles at vesicle-generation events, and to the scenario considered in the main text (left to right). The black data point corresponds to the scenario $f_{\text{pack}} = 0.5$ in Fig. 8. All data points and error bars were calculated from ten simulation replicates.

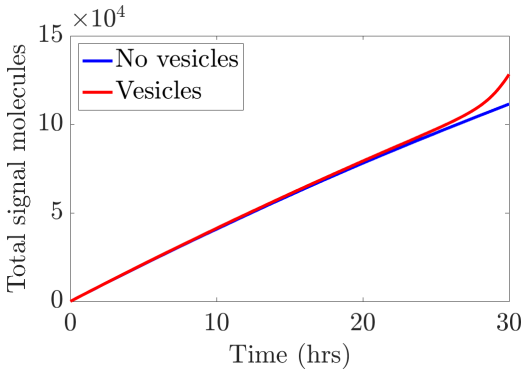

FIG. 11. Total number of signal molecules in the system as a function of time for a colony of 2000 cells, with and without vesicles. We used the same parameter values as in Fig. 3(a). Both curves were obtained by averaging over ten simulation replicates.

As discussed in the main text, vesicles are expected to protect signal molecules from decay through, for instance, interactions with enzymes in the extracellular environment. If a nonzero fraction of all signal molecules in the system is contained within vesicles, this may lead, over time, to a greater accumulation of signal molecules in the system, as compared to systems without any vesicles. The magnitude of this effect will depend on both the fraction of all signal molecules in vesicles, and on the rate of decay of the freely diffusing signal molecules. Figure 10 shows the total number of signal molecules in the system as a function of time for a colony of 2000 cells, with and without vesicles [see also Fig. 3(a)]. We find in Fig. 11 that with the parameter values used here, there is little difference in the total number of signal molecules in the system with and without vesicles up to approximately 30 h, when the first vesicle-producing cells begin to activate.

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