Emergent robustness of bacterial quorum sensing in fluid flow

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Abstract. Bacteria use intercellular signaling, or quorum sensing (QS), to share information and respond collectively to aspects of their surroundings. The autoinducers that carry this information are exposed to the external environment; consequently, they are affected by factors such as removal through fluid flow, a ubiquitous feature of bacterial habitats ranging from the gut and lungs to lakes and oceans. To understand how QS genetic architectures in cells promote appropriate population-level phenotypes throughout the bacterial life cycle requires knowledge of how these architectures determine the QS response in realistic spatiotemporally varying flow conditions. Here, we develop and apply a general theory that identifies and quantifies the conditions required for QS activation in fluid flow by systematically linking cell- and population-level genetic and physical processes. We predict that, when a subset of the population meets these conditions, cell-level positive feedback promotes a robust collective response by overcoming flow-induced autoinducer concentration gradients. By accounting for a dynamic flow in our theory, we predict that positive feedback in cells acts as a low-pass filter at the population level in oscillatory flow, allowing a population to respond only to changes in flow that occur over slow enough timescales. Our theory is readily extendable, and provides a framework for assessing the functional roles of diverse QS network architectures in realistic flow conditions.

I. INTRODUCTION

Bacteria share and respond collectively to information about their surrounding environment through the production, release and detection of small diffusible molecules called autoinducers, in a process termed quorum sensing (QS). In QS systems, the individual bacterial expression of genes relevant to the community is promoted when autoinducers accumulate to a threshold concentration, typically associated with an increasing cell density [1]. Population-level behaviors exhibited in QS-activated states include bioluminescence [2, 3], virulence factor production [4], modified mutation rates [5], biofilm and aggregate formation [6, 7], and biofilm dispersal [8]. As autoinducers diffuse between cells, they are often subject to complex and fluctuating features of their environment, such as extracellular matrix components [9], interference by other bacterial species (or the host organism), and external fluid flow. Recent research has started to show how such environmental factors are closely linked to the QS response, building on foundational knowledge gained from studying well-mixed laboratory cultures [10–12]. However, improving our understanding of the functional role of QS systems requires understanding how these systems promote appropriate population-level phenotypes in realistic bacterial environments.

Fluid flow is ubiquitous in a diverse range of bacterial habitats from rivers, lakes, and medical devices to the host teeth, gut, lungs, and nasal cavity [13]. In addition to its mechanical effects on the structure of cell populations [14–18], external fluid flow has been found to have a strong influence on the transport of relevant chemicals including nutrients [8, 19], antibiotics during host treatment [20, 21], and QS autoinducers [22–25]. In strong enough external flow, autoinducer removal has been found to suppress QS activation entirely [24, 25], raising the question of when QS systems are able to respond to increasing cell density in environments that promote high mass transfer [26–29]. Advective transport of autoinducers can also induce chemical gradients within a population by causing a local build-up of autoinducers, both downstream and within regions sheltered from the external fluid through complex topography or an extracellular matrix [25]. Thus, flow can drive heterogeneous phenotypes in genetically identical populations [30]. However, although these important macroscopic aspects of the QS response to fluid flow have been observed experimentally [22–25] and in simulations [31–37], it remains unclear how the properties of QS genetic networks, which contain significant differences across species [38], control emergent population-level responses in realistic steady or intermittent flow conditions.

Here, we show how positive feedback, a common feature of QS genetic circuits [38], promotes a robust population-level response to spatiotemporally varying external fluid flow. First, we establish the conditions required for the emergence of population-level QS activation in steady flow. By combining simulations and a systematic asymptotic analysis, we identify two key dimensionless parameter groups that characterize the physical, geometric and kinetic aspects of the system. We find that these parameter groups are linked by an algebraic relationship at the onset of QS activation. Our results illustrate how, for QS systems that exhibit positive feedback,

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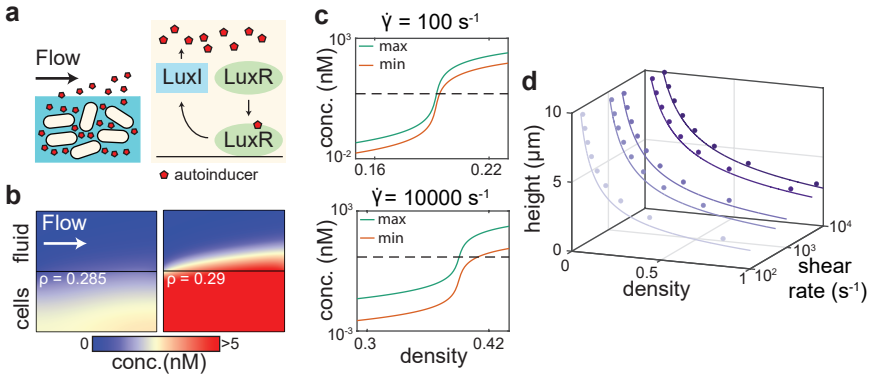

FIG. 1: We identify the required conditions for the onset of quorum sensing (QS) activation in a LuxIR-type system in steady flow. a) We model a population of cells, and an external fluid flow above the population (left). Inside cells, we model a LuxIR-type genetic circuit with positive feedback [11, 38] (right). b) In steady simulations for an imposed uniform shear flow, if the density is below a critical value, the system remains inactivated (left). If the density rises just above this critical value, positive feedback causes robust population-level QS activation (right). c) The maximum and minimum autoinducer concentrations inside a population rise drastically around the critical density in steady simulations (the dashed line corresponds to the activation threshold). d) The simulations show that the critical density is larger for a larger shear rate, and smaller for a larger cell population (dots). Lines show the predicted critical density from Eqs. (2)-(4). All kinetic parameters in these simulations are given the values listed in Table S1.

the required conditions depend primarily on the region of the population where the local ratio of advective to diffusive transport at the interface between the population and the external flow is lowest. Furthermore, we find that by combining multiple QS signals, a population can infer both cell density and external flow conditions. By accounting for a dynamic flow in our model, we find that positive feedback acts as a low-pass filter to oscillations in the shear rate with period shorter than a critical time that we calculate, allowing a population to respond only to its average external flow conditions. Overall, our findings suggest that positive feedback allows QS systems to act as spatiotemporally non-local sensors of fluid flow.

II. RESULTS

Population-level theory for QS in flow

To understand how genetic circuits in individual cells affect population-level bacterial signaling, we investigated an archetypal quorum sensing (QS) circuit in gram-negative bacteria called a LuxIR system (Fig. 1a; Methods). In this system, autoinducers in cells bind to a cognate LuxR protein and the bound autoinducer-LuxR dimer promotes the transcription of downstream genes. The system exhibits a positive feedback loop through the presence of an autoinducer synthase, LuxI, whose expression is promoted by the bound dimer [11, 38]. Thus, to summarize, as the concentration of autoinducers in a cell increases, there is an increase in the number of bound dimers. Consequently, this promotes the production of LuxI, which further increases the production of autoinducers.

We modeled the concentration of autoinducers, LuxR, LuxI, and bound autoinducer-LuxR dimers in a population of cells through a locally-averaged set of governing equations [39, 40]. The important cell-scale information is captured through the local volume density of cells ρ (Methods). We consider the scenario where cells are embedded in extracellular matrix [16] (matrix generation precedes QS activation in species including *Vibrio cholerae* [8] and *Pseudomonas aeruginosa* [24]) over which a fluid flows. As such, the fluid flow imparts a shear stress (which may vary in space and time) to the upper boundary of the cell layer. We neglect growth-induced flows inside the cell layer because their timescales are typically much slower than those of diffusion and external flow [17, 41]. Thus, our problem consists of two coupled domains: the external flow region where an advection-diffusion equation holds for the autoinducer concentration; and the cell population region where a diffusion-reaction equation holds for the autoinducer concentration, and reaction equations hold for the concentrations of the proteins and dimers inside cells in the LuxIR system. Autoinducer decay was accounted for on both domains; see Methods for the full governing equations. A flow applying a typical shear rate $\dot{\gamma}$ to a cell population of height H containing autoinducers with diffusion coefficient D introduces the Péclet number

$$\mathrm{Pe} = \dot{\gamma} H^2 / D. \tag{1}$$

The Péclet number is a key control parameter we use to investigate how external flow affects QS in cell populations.

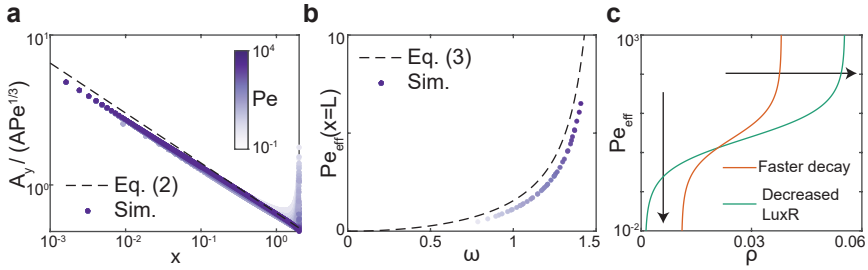

FIG. 2: We identify and analyze an algebraic relationship linking flow, biomass and kinetics at the onset of quorum sensing (QS) activation. a) For steady simulations across a wide range of shear rates, when the calculated effective Péclet number at the cell population boundary, A_y/A, is scaled by $\mathrm{Pe}^{1/3}$, the simulation data collapse onto the curve $0.65/x^{1/3}$ (dashed line), as predicted in Eq. (2). Here A is the autoinducer concentration and y is the co-ordinate perpendicular to the flow. Simulations correspond to those in Fig. 1d. b) When plotted against the appropriate non-dimensional variables identified in Eqs. (2)-(3), the simulations from Fig. 1d collapse onto the curve defined by Eq. (4) (see also Fig. S4). c) An illustration of how cells can measure an increase in density (horizontal arrow) or a decrease in shear rate (vertical arrow) by measuring concentrations of two different autoinducers and identifying which signal activates first. The signal corresponding to the red line decays 20 times faster and has a LuxR production rate 2 times faster than the signal corresponding to the green line. Parameters are given the values in Table S1, with a population height of 500 μm, a LuxR production decreased by a factor of 10, and LuxR and LuxI decay increased by a factor of 10, so that autoinducer decay approximately balances with positive feedback.

QS activation in steady flow

First, we performed numerical simulations of the governing equations in the finite-element computational software COMSOL Multiphysics for an imposed spatially uniform shear flow, which we assumed to be free of autoinducers far upstream (see Fig. S1 for a full description of the numerical procedure). This incorporates a very wide range of laminar flows in simple geometries owing to the large Schmidt number ($\mathrm{Sc} = \nu/D > 1000$, where ν is the kinematic viscosity and D is the diffusion coefficient) of autoinducers in water, so that the flow profile can be linearized in the mass-transfer boundary layer. We performed steady simulations to understand the conditions in which it is possible for a population to enter a QS-activated state for typical kinetic and physical parameter values (summarized in Table S1). The results show that a strong flow can entirely suppress QS activation by removing autoinducers from the population boundary (Fig. 1b). However, above a critical cell density ρ_c, the cell population is able to exhibit QS activation through the positive feedback present in the system. At steady state in this regime, the domain becomes flooded with autoinducers (Fig. 1b), which increase in concentration by several orders of magnitude throughout the population. This change occurs over very small changes in cell density (Fig. 1c). In larger cell populations and weaker external flows, the critical density is smaller owing to the reduced mass transfer of autoinducers out of the population (Fig. 1d).

To understand the general principles that guide how the various kinetic, physical and geometric parameters determine ρ_c, we analyzed the system of equations for a thin cell layer, where diffusion through the cell layer in the direction of flow is much less important than diffusion in the direction normal to the surface of the cell layer [42]. In this systematically reduced model, the entire effect of the external flow region on the autoinducer concentration A within the cell population is reduced to an effective Robin boundary condition on the surface of the cell layer. To derive this condition, we constructed a similarity solution for A in the mass-transfer boundary layer in the external fluid (see Supporting Information). This yielded an effective Péclet number, $\mathrm{Pe}_{\mathrm{eff}}$, which quantifies the local ratio of advective to diffusive transport at the position x (where the x-axis is directed with the flow and $x = 0$ corresponds to the upstream edge of the population). Our analysis predicts the effective Péclet number to be

$$\mathrm{Pe}_{\mathrm{eff}} = \frac{\Gamma(2/3)\mathrm{Pe}^{1/3}}{3^{2/3}x^{1/3}} \approx 0.65\frac{\mathrm{Pe}^{1/3}}{x^{1/3}}, \qquad (2)$$

which agrees well with simulation results (Fig. 2a) outside a small diffusive boundary layer of thickness $\mathrm{O}(\mathrm{Pe}^{-1/2})$ at the downstream end of the cell population (see Fig. S3).

The steady thin-film governing equations admit a solution for A that satisfies an ordinary differential equation, which we analyze through the method of matched asymptotic expansions (see Supporting Information). Based on the expected orders of magnitude of the parameter values (see Table S1), we exploit the physiologically relevant limit $K \gg 1$ and $\Lambda \gg 1$, where K and Λ are defined in Eqs (12)-(13), and represent non-dimensional measures of the strength of the feedback in the system (see Methods). Our analysis demonstrates that, for a fixed $\mathrm{Pe}_{\mathrm{eff}}$, the system exhibits an imperfect transcritical bifurcation at a critical density, which marks an orders of magnitude increase in A owing to a drastic increase in positive feedback. We identify this point as the critical density ρ_c above which the population exhibits QS activation,

which reveals the algebraic relationship

$$\omega \tan \omega = \mathrm{Pe}_{\mathrm{eff}} \quad \text{at } \rho = \rho_c \tag{3}$$

where

$$\omega := \sqrt{\frac{\rho r \Lambda}{K} - \kappa}. \tag{4}$$

Here r and κ are non-dimensional measures of LuxR production and autoinducer decay, respectively (see Methods). In Eq. (3), the effect of flow is captured in $\mathrm{Pe}_{\mathrm{eff}}$ and the effect of the kinetic parameters at the population level is captured in the single non-dimensional parameter group ω.

To compare the predictions from our systematically reduced model to the simulation results, we note that the onset of QS activation will occur at the lowest effective Péclet number at the boundary of the cell population. Because $\mathrm{Pe}_{\mathrm{eff}}$ decreases in x (see Eq. 2), we ignore the small downstream boundary layer for simplicity and assume that $\mathrm{Pe}_{\mathrm{eff}}$ is minimized at the downstream end of a cell population of length L. Therefore, we insert $x = L$ into Eq. (2) to predict $\mathrm{Pe}_{\mathrm{eff}}$ for a general population of cells in a uniform shear flow. Plotting our simulation results against the non-dimensional parameter groups $\mathrm{Pe}_{\mathrm{eff}}$ and ω (defined in Eqs. 2 and 4, respectively) demonstrates a remarkable collapse onto the predicted curve Eq. (3) for a wide range of typical kinetic, geometric and physical parameters (Fig. 2b, Fig. S4), despite the assumptions in our thin-film reduction. We note that we slightly underestimate $\mathrm{Pe}_{\mathrm{eff}}$ (and therefore ρ_c) due to the thin diffusive region at the downstream end of the population; an improvement would require a full spatial asymptotic analysis of the problem. This collapse onto Eq. (3) can be 'unwrapped' to calculate the critical conditions for activation for a QS network with a given set of kinetic parameters. For example, we found that, by combining two signals with different autoinducer decay rates and production rates of their cognate LuxR protein, a bacterial population can respond differently to either an increasing density or a decreasing shear rate; a robust measurement requires autoinducer decay to have a similar order of magnitude as positive feedback strength (Fig. 2c).

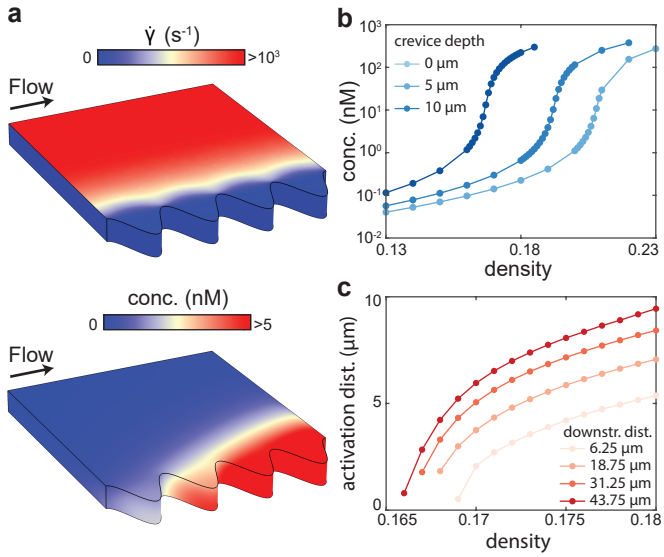

FIG. 3: In complex geometries, positive feedback causes robust QS activation based on the region with the lowest effective Péclet number. a) We simulated steady flow in a channel with crevices in its sidewall; a cell population coats the channel floor. Top: The shear rate applied by the flow to the top of the cell population is lower in the crevices (no shear is applied to the sides of the cell population). Bottom: The autoinducer concentration in the cell population is higher in the crevices and downstream. Kinetic parameters correspond to those in Table S1, with $\rho = 0.168$ and a crevice depth of 10 μm , which is twice the height of the population (see Fig. S1 for further details of the simulation geometry and boundary conditions). b) Steady simulations show that QS is activated at lower densities in populations in channels with deeper crevices. The maximum autoinducer concentration in the population, which occurs in the most downstream crevice (see panel a), is plotted. c) Steady simulations show that for a larger density, a larger region of the population is activated. The density is plotted against the QS-activated distance along the line transverse to the center of each of the four crevices; the downstream distance of each crevice from the upstream edge of the population is labeled. The QS-activated region is defined as the region for which $A > 5$ nM, which we take to be the activation threshold. All kinetic parameters were given the values listed in Table S1.

QS activation in complex geometries

In complex geometries there will be regions of low shear on the cell layer surface, on which the local effective Péclet number Eq. (2) will be reduced. Eq. (3) predicts that the global ρ_c will be lowered in such cell populations, in agreement with a recent experimental study in which QS activation was found to be promoted in crevices or pores [25]. To further understand the local and global effects of complex geometries on QS, we performed simulations of the 3D governing equations in channels which mimic typical host environments such as intestinal crypts and tooth cavities. The channels contain crevices that extend in the horizontal direction transverse to a pressure-induced flow over a cell population that coats the channel floor (Fig. 3a; Fig. S1). We found that ρ_c is reduced in channels with crevices, by an amount that depends on crevice depth (Fig. 3b), in agreement with experimental findings [25]. Furthermore, we found that once QS is activated in the crevices, diffusion of autoinducers activates further regions of the cell population outside the crevices, particularly downstream (Fig. 3c). This activation region can extend for lengths far beyond the size of the crevices themselves, even in conditions for which QS activation would be precluded completely in a simple channel (Fig. 3b,c). This demonstrates that local geo-

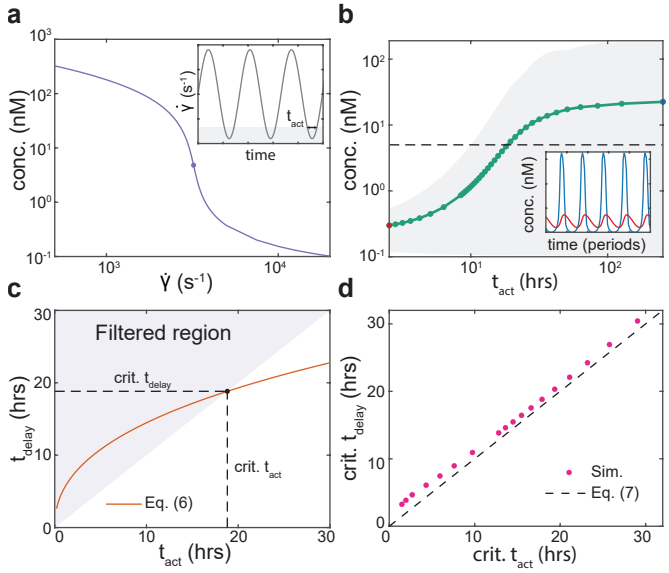

FIG. 4: We identify the required conditions for QS activation in an oscillating flow. a) In steady simulations of a population of fixed density in an imposed uniform shear flow, the maximum concentration of autoinducers rises drastically when the shear rate is below its critical value. In the following simulations we oscillate the shear rate across this critical value with a fixed mean and amplitude; the oscillations are defined by the time $t_{\rm act}$ for which the system is in the QS activated region of parameter space (inset). b) Simulations were performed with oscillations in the shear rate with mean $\dot{\gamma} = 10000$ s^{-1} and amplitude $\dot{\gamma} = 9000$ s^{-1}. For oscillation time periods slower than a critical time, corresponding to a critical $t_{\rm act}$, the mean (green line) and range (grey area) of the maximum autoinducer concentration throughout each oscillation rise drastically (see also Fig. S5). Oscillations in the autoinducer concentration are filtered out for $t < t_{\rm act}$, but not for $t > t_{\rm act}$ (inset). c) We predict a critical value of $t_{\rm act}$ by setting it to be equal to the predicted delay time, Eq. (6). d) In 1D simulations of the thin-film equations, where the Péclet number is directly controllable, we found that the critical $t_{\rm act}$ collapses onto Eq. (7) when plotted against the predicted critical delay time from panel c. The critical $t_{\rm act}$ was defined as the oscillation for which the maximum in the autoinducer concentration first rose above the threshold value of 5 nM. All kinetic parameters in these simulations were given the parameter values in Table S1.

metric complexities can have highly non-local effects on QS activation through positive feedback.

QS activation in unsteady flow

To understand the transient process of QS activation in unsteady flows, which are common in bacterial habitats such as the lungs and medical devices, we performed simulations of the dynamic governing equations for spatially uniform flows with a sinusoidally oscillating shear rate. Each oscillating flow is characterized by the time $t_{\rm act}$ during an oscillation period for which the system is in the 'QS activation region' of parameter space,

$$t_{\rm act} = \int_V {\rm d}t \tag{5}$$

where V is the set of times such that ${\rm Pe}_{\rm eff}(t) < {\rm Pe}_{\rm eff}(t_c)$ over one oscillation, and t_c is the time at which the effective Péclet number ${\rm Pe}_{\rm eff}$ falls below its critical value identified in Eq. (3) (see inset of Fig. 4a). For the range of shear rates spanned by the oscillations, the steady solution for autoinducer concentration varies over orders of magnitude as the system passes through the critical ${\rm Pe}_{\rm eff}$ (Fig. 4a). However, in the dynamic simulations, for a fixed amplitude and mean oscillation, this range is only achieved for long enough oscillation periods (i.e. larger $t_{\rm act}$). Surprisingly, for shorter oscillation periods (i.e. smaller $t_{\rm act}$), the autoinducer concentration remains below the QS-activation threshold; we note that throughout these simulations, $t_{\rm act}$ remains well above the diffusive timescale. We found that there is a critical oscillation period, and that as the period increases over this critical value, the mean (and maximum) concentration of autoinducers increases over several orders of magnitude (Fig. 4b).

To explore how this observation depends on the LuxIR system kinetics, and to determine whether it is a general property of the system, we performed a dynamic analysis of the thin-film equations as ${\rm Pe}_{\rm eff}$ passes below its critical value at $t = t_c$ and the system enters the 'QS activation region' of parameter space. We found that there is a critical slowing down due to the 'ghost' of an imperfect transcritical bifurcation in the system [43–45], which marks the onset of QS activation (see Supporting Information). This slower timescale introduces a 'delay time', $t_{\rm delay}$, which determines the time $t = t_c + t_{\rm delay}$ at which dynamic QS activation occurs if the effective Péclet number remains below its critical value, i.e. if ${\rm Pe}_{\rm eff}(t) < {\rm Pe}_{\rm eff}(t_c)$ for $t > t_c$. The delay time depends on the system kinetics through two non-dimensional parameter groups ν_1 and ν_2, (defined in Eq. 14; see Methods), and on the imposed external flow through the time-derivative ${\rm Pe}'_{\rm eff}(t_c)$ of the effective Péclet number as it passes below its critical value:

$$t_{\rm delay} = \nu_1 \sqrt{\frac{\log \nu_2 {\rm Pe}'_{\rm eff}(t_c)}{{\rm Pe}'_{\rm eff}(t_c)}}. \tag{6}$$

Interestingly, this result suggests that for an oscillating flow, if $t_{\rm delay}$ is longer than $t_{\rm act}$, then $t_{\rm delay}$ is long enough to prevent the onset of dynamic QS activation by flow entirely (Fig. 4c). Thus, assuming an oscillating ${\rm Pe}_{\rm eff}$ that enters the QS activation region of parameter space for a period $t_{\rm act}$ during each oscillation, we identify that at the critical oscillation period

$$t_{\rm act} = t_{\rm delay}. \tag{7}$$

Eqs. (6)-(7) predict that for a flow oscillating over a long enough time period, such that $t_{\rm act} > t_{\rm delay}$, the QS sys-

tem passes through cycles of dynamic activation and deactivation. Conversely, for a flow oscillating over a short enough time period, such that $t_{\mathrm{act}} < t_{\mathrm{delay}}$, the system is predicted to remain in the QS-inactivated state. Simulations of the governing thin-film equations, in which the Péclet number and its derivatives are directly controllable, show that for a wide range of oscillating Péclet numbers, the calculated properties for the onset of dynamic QS activation collapse onto the curve defined by Eqs. (6)-(7) (Fig. 4d).

In experiments, and in our simulations of the governing equations with an imposed flow, it is not possible to control the effective Péclet number and its derivatives directly. However, we can make an order of magnitude estimate of the required conditions for the onset of dynamic QS activation for a sinusoidally oscillating flow with a time period T_p as follows. At onset, $\mathrm{Pe}'_{\mathrm{eff}}(t_c) = O(1/T_p)$ and $t_{\mathrm{act}} = O(T_p)$. Combining Eq. (6) and (7) and neglecting the effect of the logarithmic term in Eq. (6) (which we expect to have a lesser effect than the algebraic terms) yields an estimate of $T_p = O(\nu_1^2)$ at the onset of dynamic QS activation. For the typical kinetic parameters used in our simulations (see Table S1), this suggests a critical oscillation period of approximately 10 hours, which is in agreement with our simulation results for a wide range of oscillating flows (Fig. 4b,d; Fig. S5).

III. DISCUSSION

This study demonstrates how positive feedback in the LuxIR system, an archetypal bacterial quorum sensing (QS) genetic circuit, promotes a robust population-level response in spatiotemporally varying flow conditions. Because QS systems measure the concentration of passively transported autoinducers, even simple fluid flows generate concentration gradients which can cause phenotypic gradients within a population. Our results show that positive feedback in QS genetic architectures allows bacteria to overcome flow-induced autoinducer gradients at the population level for a wide range of conditions that represent flows encountered in bacterial habitats such as lakes, rivers, and hosts (Figs. 1, 3). The key physical determinant of the onset of QS activation for a population is the minimum value of the effective Péclet number $\mathrm{Pe}_{\mathrm{eff}}$ (see Eq. 2), which quantifies the local advective to diffusive transport at the surface where the population meets the external fluid. Furthermore, a systematic reduction of the governing equations via a thin-film model reveals that for a given $\mathrm{Pe}_{\mathrm{eff}}$, the critical population density (or size) for the onset of QS activation is determined by a single dimensionless kinetic parameter group ω (see Eq. 4). A compact relationship between these two parameter groups, Eq. (3), links the physical, geometric and kinetic parameters at the onset of QS activation (Fig. 2). Through its transparent dependence on these system parameters, Eq. (3) explains how QS activation is promoted in larger or denser populations, in bacteria with QS architectures with stronger positive feedback or with smaller autoinducer-LuxR dissociation constants [46], and in populations subject to weaker external flow.

The dependence of the critical density on the flow conditions raises the question of whether bacterial QS systems in fluid flow respond to increasing cell density, decreasing mass transfer, or a combination of these factors [28]. By calculating the critical conditions for QS activation for two sets of kinetic parameters using Eqs. (3)-(4), we suggest that a bacterial population can respond differently to a decreasing shear rate and an increasing density by integrating information from multiple signals. A key finding of our analysis is that, based on Eq. (3), two signals with different decay rates can only split $(\rho, \mathrm{Pe}_{\mathrm{eff}})$ parameter space into three separate regions, because the critical density ρ_c depends additively on the autoinducer decay rate κ. In our model, adding further fidelity to a measurement requires a difference in the QS network parameters between signals, such as a decreased production rate of the cognate LuxR protein (Fig. 2c). This result is in qualitative agreement with previous work which considered a well-mixed population subject to spatially uniform autoinducer removal by mass transfer [28]. We predict that by combining one signal with a faster decay rate and one signal with a slower LuxR production rate, a bacterial population can make a reasonable estimate about whether autoinducer concentration is increasing owing to an increasing density or a decreasing shear rate (Fig. 2c).

An individual bacterium committing to a QS-activated phenotype can incur significant individual costs, such as the generation or abandonment of important extracellular material. It is therefore often beneficial for such a commitment to be shared by the rest of the bacterial population [47]. As we have shown, positive feedback promotes a robust, population-scale QS response if appropriate conditions are met in only a local region of a cell population. While this feature of positive feedback is very useful for population-wide commitment, it has the potential downside of triggering premature QS activation in noisy or intermittent flow conditions. However, our analysis of the LuxIR system in dynamic flow conditions suggests that the positive feedback mechanism actually reduces the potential for such a premature response because the feedback itself acts as a low-pass filter at the population level. That is, in a flow oscillating with period smaller than a critical value that we calculate (still slower than the diffusion timescale), a population responds to the mean effective Péclet number, rather than exhibiting quasi-steady oscillations in QS activation and inactivation (Fig. 4). In such conditions, a growing population's QS system would be expected to activate eventually through its increasing density (growth is also associated with an activation delay time but for typical parameters it is of the same order of magnitude as the doubling time; see Fig. S6). The critical time period depends on the nature of the flow

oscillation and the system parameters through Eq. (6), which manifests due to a critical slowing down induced by an imperfect transcritical bifurcation in the system; such bottlenecks are a universal feature of dynamical systems near critical points [43–45]. This result suggests that population-level low-pass filtering via positive feedback complements other previously identified sources of noise-filtering, or time-averaging [48], in QS systems such as slow autoinducer-LuxR unbinding [49] and diffusional dissipation [50, 51].

Our findings allow us to interpret typical QS network features in a manner that accounts for their expected response to spatiotemporal variations in flow. Our analysis suggests that bacterial species with positive feedback in their QS network, such as *P. aeruginosa*, use QS as a non-local spatiotemporal sensor of flow conditions and cell density; we expect these species to exhibit QS activation as a large proportion of the population, if the required flow conditions persist for long enough in any region of the population. Conversely, we expect species with no or negative feedback in their QS network, such as *V. cholerae*, to use QS as a local sensor of flow conditions and cell density; we expect them to exhibit QS activation quickly and locally if the concentration of autoinducers reaches the activation threshold in a region of the population.

To conclude, our key results are summarized in Eqs. (2)-(7). These algebraic relationships capture the required conditions for the emergence of robust, population-level QS activation in a spatiotemporally varying fluid flow, for systems that exhibit positive feedback in their QS network architecture. We have confirmed their validity against simulations in a wide range of flows representing those encountered in typical bacterial habitats. Our theory demonstrates how QS genetic architectures play a key role in determining the population-level functional response of bacterial intercellular signaling systems in complex environments.

IV. METHODS

A. Governing equations

Inside the cell population, the governing equations are

$$\begin{aligned} \frac{\partial \tilde{A}}{\partial \tilde{t}} &= \tilde{\nabla} \cdot \left(\tilde{D} \tilde{\nabla} \tilde{A} \right) + \rho \tilde{f}_A(\tilde{A}, \tilde{I}, \tilde{R}, \tilde{C}), \\ \frac{\partial \tilde{I}}{\partial \tilde{t}} &= \tilde{f}_I(\tilde{I}, \tilde{C}), \quad \frac{\partial \tilde{R}}{\partial \tilde{t}} = \tilde{f}_R(\tilde{A}, \tilde{R}, \tilde{C}), \\ \frac{\partial \tilde{C}}{\partial \tilde{t}} &= \tilde{f}_C(\tilde{A}, \tilde{R}, \tilde{C}), \end{aligned} \tag{8}$$

where ρ is the volume fraction of cells, $\tilde{A}$ is the concentration of autoinducers, $\tilde{I}$ is the concentration of LuxI, $\tilde{R}$ is the concentration of LuxR, and $\tilde{C}$ is the concentration of autoinducer-LuxR dimers. The reaction terms in the system (Fig. 1a) are

$$\begin{aligned} \tilde{f}_A(\tilde{A}, \tilde{I}, \tilde{R}, \tilde{C}) &= \tilde{q} + \tilde{\lambda}\tilde{I} - \tilde{k}_+ \tilde{A}\tilde{R} + \tilde{k}_- \tilde{C} - \tilde{\kappa}\tilde{A}, \\ \tilde{f}_I(\tilde{I}, \tilde{C}) &= \tilde{\mu}\tilde{C} - \tilde{\alpha}\tilde{I}, \\ \tilde{f}_R(\tilde{A}, \tilde{R}, \tilde{C}) &= \tilde{r} - \tilde{k}_+ \tilde{A}\tilde{R} + \tilde{k}_- \tilde{C} - \tilde{\beta}\tilde{R}, \\ \tilde{f}_C(\tilde{A}, \tilde{R}, \tilde{C}) &= \tilde{k}_+ \tilde{A}\tilde{R} - \tilde{k}_- \tilde{C} - \tilde{\gamma}\tilde{C}. \end{aligned} \tag{9}$$

The meanings and typical orders of magnitude of each dimensional parameter are listed in Table S1. Here, for simplicity we have assumed that mRNA concentrations are quasi-steady, and have linearized the activation of LuxI by the autoinducer-LuxR dimers [52] (see Fig. S2 for a discussion of how saturation in promoter occupancy of the transcription factor affects QS activation in flow). Note also that we do not consider the basal expression of LuxI, which is expected to be small [53], because its only effect is to slightly change the concentration of autoinducers before activation. Outside the cell population, the governing equations are

$$\nabla \cdot \tilde{\boldsymbol{u}} = 0, \quad \frac{\partial \tilde{\boldsymbol{u}}}{\partial \tilde{t}} + \tilde{\boldsymbol{u}} \cdot \nabla \tilde{\boldsymbol{u}} = -\frac{1}{\rho_w} \nabla p + \nu \nabla^2 \tilde{\boldsymbol{u}}, \tag{10}$$

$$\frac{\partial \tilde{\mathcal{A}}}{\partial \tilde{t}} = \tilde{\nabla} \cdot \left(\tilde{D} \tilde{\nabla} \tilde{\mathcal{A}} - \tilde{\boldsymbol{u}} \tilde{\mathcal{A}} \right) - \tilde{\kappa} \tilde{\mathcal{A}}, \tag{11}$$

where $\tilde{\mathcal{A}}$ is the autoinducer concentration in the fluid, $\tilde{\boldsymbol{u}}$ is the velocity field of the fluid, and ρ_w and ν are the fluid density and kinematic viscosity, respectively, which we take to be those of water. We assume that the flow is free of autoinducers far upstream, and apply continuity of autoinducer concentration and concentration flux at the interface between the cell population and the flow. Full technical details of the boundary conditions, the non-dimensionalisation of the problem, and the procedures for the asymptotic and numerical solution of the governing equations are given in the Supporting Information. All code required to generate the simulation results is available on Github at https://github.com/philippearce/quorum-flow (requires Comsol 5.5 license, Matlab license, and Comsol LiveLink with Matlab License).

B. Non-dimensional parameters

The non-dimensional parameters, defined in terms of the dimensional parameters in Eqs. (8)-(9), are

$$\begin{aligned} q &= \frac{\tilde{q}\tilde{H}^2}{\tilde{A}_0 \tilde{D}}, \quad r = \frac{\tilde{r}\tilde{H}^2}{\tilde{A}_0 \tilde{D}}, \quad \lambda = \frac{\tilde{\lambda}\tilde{H}^2}{\tilde{D}}, \\ \mu &= \frac{\tilde{\mu}\tilde{H}^2}{\tilde{D}}, \quad k_+ = \frac{\tilde{k}_+ \tilde{A}_0 \tilde{H}^2}{\tilde{D}}, \quad k_- = \frac{\tilde{k}_- \tilde{H}^2}{\tilde{D}}, \\ \alpha &= \frac{\tilde{\alpha}\tilde{H}^2}{\tilde{D}}, \quad \beta = \frac{\tilde{\beta}\tilde{H}^2}{\tilde{D}}, \quad \gamma = \frac{\tilde{\gamma}\tilde{H}^2}{\tilde{D}}, \\ \kappa &= \frac{\tilde{\kappa}\tilde{H}^2}{\tilde{D}}, \end{aligned} \tag{12}$$

where $\tilde{H}$ is the height of the cell population and $\tilde{A}_0$ is the threshold concentration of autoinducers for QS activation. These parameters can be combined into the parameter groups

$$K = \frac{\beta(k_- + \gamma)}{k_+ \gamma}, \quad \Lambda = \frac{\lambda\mu}{\alpha\gamma}, \tag{13}$$

and

$$\begin{aligned} \nu_1 &= \frac{\sqrt{2I_2\xi}}{\cos\omega}, \quad \nu_2 = \frac{I_2 \xi K^2 \cos^2\omega}{\rho_c^2 q r I_1 I_3 \Lambda}, \\ \xi &= 1 + \frac{r\rho_c\Lambda}{K}\left(\frac{1}{\alpha} + \frac{1}{\gamma + k_-}\right), \end{aligned} \tag{14}$$

where

$$I_n = \int_0^1 \cos^n \omega z \, \mathrm{d}z, \tag{15}$$

and ω is defined in Eq. (4)

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