A stabilized microbial ecosystem of self-limiting bacteria using synthetic quorum-regulated lysis

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Microbial ecologists are increasingly turning to small, synthesized ecosystems1-5 as a reductionist tool to probe the complexity of native microbiomes6,7. Concurrently, synthetic biologists have gone from single-cell gene circuits8-11 to controlling whole populations using intercellular signalling12-16. The intersection of these fields is giving rise to new approaches in waste recycling17, industrial fermentation18, bioremediation19 and human health16,20. These applications share a common challenge, well-known in classical ecology21,22—stability of an ecosystem cannot arise without mechanisms that prohibit the faster-growing species from eliminating the slower. Here, we combine orthogonal quorum-sensing systems and a population control circuit with diverse self-limiting growth dynamics to engineer two ‘ortholysis’ circuits capable of maintaining a stable co-culture of metabolically competitive Salmonella typhimurium strains in microfluidic devices. Although no successful co-cultures are observed in a two-strain ecosystem without synthetic population control, the ‘ortholysis’ design dramatically increases the co-culture rate from 0% to approximately 80%. Agent-based and deterministic modelling reveal that our system can be adjusted to yield different dynamics, including phase-shifted, antiphase or synchronized oscillations, as well as stable steady-state population densities. The ‘ortholysis’ approach establishes a paradigm for constructing synthetic ecologies by developing stable communities of competitive microorganisms without the need for engineered co-dependency.

To engineer a stable co-culture of two competitive bacterial strains, we first characterized the dynamics of a small library of quorum-sensing (QS) components (Supplementary Fig. 1a–c). This was achieved by evaluating different components of natural QS systems to identify receptor–promoter pairs and signals (acyl homoserine lactone, AHL) that yield the desired characteristic upon combination (Supplementary Fig. 1d)23. From a range of possible configurations (Supplementary Fig. 2b), we identified that the Lux and Las systems were suitable for one-way orthogonal signalling, and the Lux and Rpa systems were suitable for two-way orthogonal signalling (Supplementary Fig. 1e–g). We used these components to design synchronized lysis circuits (SLCs)16 in two bacterial strains, where each strain is programmed to lyse upon reaching a critical population density.

To understand how an ecosystem harbouring an SLC can be altered, we established a range of possible self-limiting dynamics for the circuit (Fig. 1a,b). The circuit exhibits oscillations characterized by periodic lysis events, which are driven by activation of the Lux-controlled positive feedback loop upon reaching a quorum threshold of AHL, as seen in earlier work16. A lysis event reduces the population dramatically, and a few survivors resume the process, starting again below the quorum threshold. In microfluidic devices, the superfoldering green fluorescent protein (sfGFP) reports the activation state of the circuit in this oscillatory state (Fig. 1c and Supplementary Video 1). We also discovered a constant lysis state characterized by a steady state in which growth and lysis are approximately balanced, and the stable ‘on’ state of the circuit is evidenced by the constant production of sfGFP (Fig. 1d and Supplementary Video 2). Tuning the degradation efficiency of the activator LuxI by changing its ssrA degradation tag, we demonstrated a bifurcation in the lysis dynamics of the population between these two states. In a deterministic model of the circuit (Fig. 1b), stronger enzymatic degradation of LuxI is represented by a lower basal signal production rate $a_0$ (see Methods for details). Consistently, the oscillatory lysis behaviour was observed for the highest level of activator degradation (Fig. 1e,f), dampened oscillations were observed at a lower level of degradation (Fig. 1g,h), and constant lysis behaviour was observed for the lowest levels of degradation (Fig. 1i,j). The SLC therefore exhibited two main modes of dynamic lysis with respect to changes in circuit parameters.

To build a synthetic ecosystem of two orthogonal SLC strains, we used the previously built circuit based on the Lux QS system and constructed a new circuit with the Rpa system. The Rpa system had RpaR in place of LuxR and an ssrA tagged RpaI in place of LuxI (Fig. 2a). These strains are called Lux-CFP and Rpa-GFP, respectively, for convenience. Both strains’ gene expression is controlled by the PluxI promoter for consistency, considering p-coumaroyl-HSL-bound RpaR can activate PluxI at about 90% the efficiency of AHL-bound LuxR while still avoiding crosstalk (Supplementary Fig. 2b)24. Although these strains are in the same bacterial host, when started from equal densities in batch culture, Rpa-GFP shows a significant growth advantage over Lux-CFP (Fig. 2b). Because of this growth advantage, a 1:1 mixture of these strains in a batch culture (with or without the lysis gene) is primarily taken over by the faster-growing Rpa-GFP strain by the time the strains reach stationary phase (Fig. 2c). However, if the slower-growing Lux-CFP strain is enriched 100 times more than the Rpa-GFP strain, the population-stabilizing effects of the lysis circuit become evident. Without the lysis gene, the mixture is taken over by the Lux-CFP strain, but with the lysis gene, the population ratio over the initial 10 h keeps close to a 1:1 ratio. The ‘ortholysis’ strategy thus showed promise in batch co-culture.

We then grew the strains in microfluidic devices, with a seeding ratio of 1:10 (Rpa-GFP to Lux-CFP) optimized for the new system, to examine the long-term dynamics of the co-culture. The microfluidic trap (growth chamber) harbouring the two strains without
Figure 1 | Experimental investigation into the space of the population dynamics of a self-communicating synchronized lysis circuit. a, Genetic diagram of the synchronized lysis circuit (SLC). The circuit contains a lysis plasmid and an activator/reporter plasmid. Transient production of LuxI eventually leads to an accumulation of AHL above the quorum threshold needed to activate LuxR, which begins a positive feedback loop by driving transcription off the PluxI promoters that control production of LuxI, GFP and the lysis gene ϕX174E. LuxR in this system is driven by the native LuxR promoter. b, Bifurcation to oscillations in a deterministic model of the lysis circuit. Ignoring the initial transient behaviour, minimum, maximum and mean population densities over time were determined for each parameter value. Lower αq corresponds to stronger degradation (see Methods for details). c, Video stills showing bacteria harbouring the SLC, with strong degradation of the LuxI activator (LuxI-LAA) exhibiting oscillations in a microfluidic growth chamber. Oscillations result from repeated cycles of growth, quorum threshold being reached and self-limitation by lysis activation. d, Video stills depicting bacteria harbouring the SLC with weaker degradation of LuxI (LuxI with no degradation tag), exhibiting constant lysis. Constant activation of the lysis circuit results in the continual activation of GFP as well as continuous growth and lysis events within the microfluidic chamber. e–j, Changing certain properties, such as the degradation tag of the SLC, will result in different population dynamics. Fluorescence (green) and population (black) (e,f) over time for cells harbouring the SLC with αq = 0.4 for the deterministic model (e) and LAA-tagged LuxI for a typical experimental run (f), as seen in c. Fluorescence (green) and population (black) (g,h) over time for cells harbouring the SLC with αq = 1.1 for the deterministic model (g) and TS-LAA-tagged LuxI for a typical experimental run (h). Fluorescence (green) and population (black) (i,j) over time for cells harbouring the SLC with αq = 2 for the deterministic model (i) and untagged LuxI for a typical experimental run (j), as seen in d. The video stills in c and d, as well as the fluorescence and population profiles in f, h and j, are all representative of typical microfluidic experiments with these strains.
Figure 2 | Experimental demonstration of long-term co-culture of competitive species with unequal growth rates using signal orthogonal self-lysis.

**a** Genetic diagram of a two-strain ecosystem of self-lysing Salmonella constructed with two signal orthogonal QS systems, rpa and lux. **b** Batch culture growth curves of the Lux-CFP strain alone (blue), Rpa-GFP strain alone (green), a 1:1 mixture (black) and a 1:100 (Rpa-GFP:Lux-CFP) mixture (grey), both without the lysis gene (top) and with the lysis gene (bottom). All strains were started from the same diluted density and under the same growth conditions. Width of lines represent s.d. (n = 3). **c** Batch culture population estimates of Lux-CFP and Rpa-GFP co-cultures. Rpa-GFP population is estimated as GFP fluorescence (integrated over the full length of the experiment) of the mixture, expressed as a percentage of the time-integrated GFP fluorescence of Rpa-GFP cells alone. Lux-CFP population is estimated as CFP fluorescence (integrated over the full length of the experiment) of the mixture, expressed as a percentage of the time-integrated CFP fluorescence of Lux-CFP cells alone. Error bars represent s.d. (n = 3). **d** Video stills of a representative co-culture of non-lysing Lux-CFP and Rpa-GFP strains showing eventual takeover by the green strain. **e** Video stills of a representative co-culture of the Lux-CFP and Rpa-GFP strains with the lysis plasmid. Addition of the lysis plasmid prevents either strain from taking over for the duration of the experiment. **f** Time trace of GFP and CFP fluorescence of the trap in the video shown in **d**. **g** Time trace of GFP and CFP fluorescence of the trap in the video shown in **e**. **h** Graph showing the length of co-culture for each of the 60 traps containing the non-lysing strains. **i** Graph showing the length of co-culture for each of the 60 traps containing the strains with the lysis plasmids.
the lysis gene quickly lost its co-culture and was taken over by the Rpa-GFP strain alone (Fig. 2d and Supplementary Video 3). This process was observed for 60 traps, and the time duration of the co-culture was measured over two days. All traps eventually lost their co-culture completely, with an average co-residence time of 6.5 h (Fig. 2h). However, when the two orthogonal lysis strains were grown together, most of the 60 traps maintained a co-culture for the duration of the two-day experiment (Fig. 2e and Supplementary Video 4); all traps that lost co-culture were completely taken over by the Rpa-GFP strain. Due to differences in the inherent parameters of the two QS systems, the Rpa-GFP circuit remains in the constant lysis regime and is therefore perpetually producing sfGFP. However, the Lux-CFP strain is in the oscillatory regime and remains dark until it reaches quorum threshold, and its lysis events are characterized by a punctuated burst of CFP production (Fig. 2g and Supplementary Fig. 3a–d). The bimodality of the co-residence time (either lost in the first couple hours or maintained to the end of the experiment) suggests that the small volume of these reactors and the non-deterministic loading conditions predispose some wells with very few Lux-CFP cells to stochastic loss of co-culture. Seemingly, depending on the environmental context, oscillatory strains are more susceptible to environmental perturbations than a strain in the constant lysis regime. However, the benefit of using a strain in the oscillatory lysis regime is that it leaves the possibility of engineering dynamic population profiles, which may be useful for certain applications, such as the timed delivery of two different payloads. Nevertheless, within our micro-fluidic device, the ‘ortholysis’ method is rather robust at co-culturing even competitive strains for long periods of time (Fig. 2i).

We used agent-based modelling to visually show how the ‘ortholysis’ strains might behave with different QS parameters. We first modelled a system where the QS parameters of the Rpa system were the same as the Lux system parameters used in previous studies. However, we used the experimental difference in growth whereby the Rpa-GFP strain grows at 110% the rate of the Lux-CFP strain. With the Lux-CFP strain seeded in a 10:1 ratio with Rpa-GFP, we observed that the Lux-CFP strain rapidly grew in number, while the Rpa-GFP strain remained at a constant level. This resulted in a punctuated burst of CFP production, which is characteristic of the oscillatory regime. On the other hand, when the two strains were grown together, they maintained a co-culture for the duration of the experiment, with the Lux-CFP strain eventually taking over. This observation is consistent with the experimental findings and underscores the importance of considering the inherent parameters of the QS systems when designing co-culture experiments.
Figure 4 | Prediction of synchronized lysis circuit dynamics in a dual-strain population using various communication motifs. Model-generated heat maps depicting time-averaged population ratio \((\hat{\eta}_1 - \hat{\eta}_2)/(\hat{\eta}_1 + \hat{\eta}_2)\) of Rpa-GFP (green) and Lux-CFP (blue) strains in a well-mixed, constant-flow co-culture, as a function of Rpa-GFP’s growth rate \(a_1\) against Lux-CFP’s growth rate \(a_2\). Each panel has a particular combination of lysis regimes for each of the strains, either non-lysing, lysing, or weak lysing (see Methods for details). On the left of each heatmap is the communication motif it exhibits and experimental candidate QS systems to achieve the desired signalling characteristic. These traits determine the behaviour and composition of the co-culture. The white dot on the heat map indicates the growth rate parameters selected for the time-series plots. Time-series plots show population of the Rpa-GFP and Lux-CFP strains as a function of time. a. Two non-lysing strains. b. One non-lysing strain and one lysing strain. White dashed lines indicate the growth rate at which one strain’s growth rate exceeds that of the other, even for maximum lysis activation. c. Two lysing strains with one strain having a strong response to the QS signal of the other. Cyan dashed lines indicate the region where both strains are in the oscillatory regime, and black dashed lines mark the area in which strains are self-limiting. d. Two lysing strains with one strain having a weak response to the QS signal of the other. e. Two completely orthogonal lysing strains. The rpa and lux systems could be used for this dynamic as they are signal orthogonal. f. Two completely orthogonal strains with the Rpa-GFP strain in the weak lysis regime (leading to constant lysis) and the Lux-CFP strain in the lysis regime. This is the regime corresponding to our experimental system. Oscillations in the Rpa-GFP strain’s population are imposed by the oscillatory Lux-CFP strain through volume exclusion.

respect to the Rpa-GFP strain in the model simulation, the resulting dynamics show antiphase oscillations (Fig. 3a and Supplementary Video 5). Seemingly due to volume exclusion, as shown by their fluorescence time series, the populations enter an antiphase pattern where the strains switch off growing and lysing (Fig. 3c).

We then took into consideration the innate differences between the two QS systems\(^\text{23}\) by changing several of the Rpa-GFP strain’s QS parameters in relation to the Lux parameters used. Furthermore, based on the observed phenotypic phenomenon, the probability of lysing was reduced tenfold, which allows more AHL to build up and a constant lysis dynamic to develop (Fig. 3b and Supplementary Video 6). The resulting dynamics were similar to the experimental observations, with a constantly lysing Rpa-GFP strain maintaining the majority of the population share and the Lux-CFP strain intermittently firing and lysing (Fig. 3d). To understand how these dynamics and the size of the growth container affect stability, the agent-based model was run many times under different conditions. For conditions where Lux-CFP is oscillating and Rpa-GFP is in constant lysis (lys/osc), or where both are oscillating (osc/osc), ten simulations were carried out in volumes of 20, 40 and 60 a.u. each. As the size of the space increases, so does the average residence time of the co-culture (Fig. 3e), suggesting that, as we expected, larger traps will have fewer issues with losing co-culture to stochastic events.

As evidenced by the agent-based model, our strains demonstrate only one particular dynamic of a wide-range of possibilities facilitated by QS-controlled self-lysing microorganisms with varying levels of orthogonality. We developed a reduced deterministic model to explore a wider space of possible dynamics achieved through differences in growth rates, QS systems and lysis circuit regimes. For each case, communication motifs are distinguished and suitable experimental candidate QS systems are chosen to achieve the displayed dynamics. For the two individual lysis circuits, we consider either non-lysing (no SLC), lysing (SLC) or weak lysing (less effective SLC). With two non-lysing strains, the faster-growing strain will eventually dominate the population (Fig. 4a). However, even a single strain equipped with the SLC can stabilize the co-culture, provided the non-lysing strain has the lower growth rate (Fig. 4b). In cases where both strains harbour an SLC, but there is one-way crosstalk, the strain that responds to both signals becomes entrained to the strain that only responds to its own (Fig. 4c,d). An example would be the Lux and Las systems; the Lux can respond to the Las signal, but Las is orthogonal to the Lux signal. The strength of the crosstalk determines the strength and delay of the entrainment, with strong crosstalk (Fig. 4c) exhibiting strong entrainment and weak crosstalk (Fig. 4d) showing time-delayed entrainment. In cases where each SLC operates independently, by using signal orthogonal QS systems, the most robust co-culturing is achieved where, for large ranges of growth rates, the time-averaged population ratio remains around 50/50 (Fig. 4e). If one of the strains exhibits weaker lysing dynamics, in that it has a lower probability of lysing given a quorum threshold, we obtain
reader experiments, 1 nM of 3-oxo-C6-HSL was added to all media. Plasmids were constructed using the circular cloning vector pBADm专门 for cloning or using standard restriction digest/ligation cloning16. The lux activator plasmid (Kan, ColEI) and lux-lysis plasmid (Chlor, p15A) were used in previous work from our group16,31. The Rpa and Rpal genes were obtained via PCR off the Rhodopseudomonas palustris genome (obtained through ATCC) to create the Rpa-activator and Rpa-promoter. The lux-sgFP lysis circuit alone was characterized in Escherichia coli. Co-culturing was performed with non-motive Salmonella typhimurium, SL1344.

The SLC, in both the Lux and Rpal cases, is composed of an activator plasmid and a lysis plasmid. For the circuit characterization experiments there were three variants: the first was pTD103lux-sGFP, which was used in previous work by our group16. This plasmid contains a Lux with the ssrA-LAA degradation tag (amino-acid sequence, AANDENYALAA) and sGFP, a superfolder green fluorescent protein variant15. pTD103lux (TS) sGFP was constructed by adding the TS-linker (amino-acid sequence, TS) between the ssrA-LAA tag and LuxI. pTD103lux (-LAA) sGFP was constructed by removing the ssrA-LAA tag from LuxI. For the dual lysis experiments, the Lux-CEP strain used the activator plasmid with the ssrA-LAA tagged Lux with a CFP in place of the sGFP. The Rpa-GFP strain’s activator plasmid was created by replacing LuxR with Rpal and the Lux with an ssrA-LAA tagged Rpal.

The lysis plasmids have a p15A origin of replication and a chloramphenicol resistance marker and have been previously described by our group16. The lysis gene E from the bacteriophage φX174, was provided by Lingchong You and was taken from the previously reported ePop plasmid via PCR15. The E gene was placed under the expression of the LuxR-AHL activatable LuxI promoter for both the Lux- and Rpa-GFP strains. The purpose of the construction was done using the four-component method of cloning16. Supplementary Fig. 4 and Supplementary Table 1 present maps of the plasmids used in this study.

Microfluidics and microscopy. Our group has previously described in depth the microscopy and microfluidics techniques used in this study16. In short, micrometre-scale features were baked onto silicon wafers using crosslinked photore sist. The microfluidic device resin PDMS (polydimethylsiloxane) was then poured over the wafers and solidified by baking. The PDMS, containing numerous devices, was peeled off, and individual devices were cut out from the whole. Holes were then punched into the device at their input and output, where the fluid lines would eventually plug in. After punching the devices were bonded onto glass coverslips by means of plasma activation. The devices were then put in a vacuum and the outlet loaded with cells and the inlet with medium. Vacuum pressure loaded the cells into traps, and media lines were plugged in before the cells could contaminate the upstream section of the device. The flow was then altered by changing the relative heights of the syringes (for all experiments the medium was set up to one inch above the meniscus of the waste, resulting in a low, constant hydrostatic pressure-driven flow).

All microfluidic experiments were performed in a side-trap array device as previously described16 and in all cases 0.075% Tween20 was added to the medium to prevent cells from sticking to the channels and the ports of the device. Bacteria growth chambers were 100 µm wide, 85 µm deep and ~1.6 µm in height.

For lysis characterization (Fig. 1), cells were cultured until they reached an optical density (OD) of ~0.1 (Plastibrand 1.5 ml cuvettes were used), at which point they were spun down and loaded via vacuum pressure onto the chip. The medium was LB with kanamycin and chloramphenicol.

For dual lysis and co-culturing experiments (Fig. 2), cells were cultured until they reached an optical density of ~0.1 (Plastibrand 1.5 ml cuvettes were used), and 1.5 ml was spun down and resuspended in 50 µl of medium. This concentrate was used to vacuum load the cells for single-strain experiments, or it was mixed at a 10:1 ratio (Lux-CFP:Rpa-GFP) in the co-culturing experiments before loading via vacuum pressure. The medium was LB with kanamycin and chloramphenicol.

The system used has also been described previously by our group16. In short, a Nikon Eclipse Ti epifluorescence microscope with phase-contrast-based contrast was used. The camera was a Photometrics CoolSNAP HQ2 charge-coupled device.

The acquisition software was Nikon Elements. The microfluidic devices were housed in a plexiglass incubation chamber maintained at 37 °C by a heating unit. For dual lysis and co-culturing experiments, phase-contrast images were taken at ×20 magnification with 50–200 ms exposure times. Fluorescent imaging at ×20 was performed at 300 ms for GFP, with a 30% setting on the Lumencor SOLA light source, and at 300 ms and 35% for CFP. Images were taken every 3 min for the course of the experiment (~2 days). Co-culture was determined to be lost if the fluorescence of either GFP or CFP reduced below the background fluorescence, and was checked manually for the requisite lysing CFP strain, which can drop below threshold between lysis events.

For lysis characterization (Fig. 1) we counted cells using the following strategies. For experiments where the cell population was mostly aggregated together (non-sparse population), we first estimated the average area of an individual bacterial cell and (open space between bacteria in the trap). Taking into account the pixel density of the image, we measured the area of the trap
taken up by cells using ImageJ, and divided by the average area of a bacterial cell. This value was then multiplied by \((1 - \text{void fraction})\) to yield the total estimated number of cells in the trap. Bacteria that were not close to the main group of cells were counted individually and added to the final number. For experiments where the growing population was sparse (due to the constant lysis regime), we used the Trainable Weka Segmentation plug-in for ImageJ to count cells. Plots were generated using MATLAB.

For co-culture experiments, co-culture was determined to be lost if the fluorescence of either CFP or GFP dropped below the background fluorescence, and images were then checked manually for the oscillatory lysing CFP strain, which can drop below threshold between lysis events.

Plate reader fluorescence and population estimates. For the well-plate experiments, the strains were grown in a standard Falcon tissue culture 96-well flat bottom plate with appropriate antibiotics (kanamycin only for non-lysis, and kanamycin and chloramphenicol for lysis strains). For consistency with microfluidic experiments, 1 nM of 3-oxo-C6-HSL was added to all media. We grew the bacterial strains used in Fig. 2b in 4 ml cultures to an optical density of 0.15 before adding 10 µl of this culture to 10 ml of fresh LB with appropriate antibiotics and added HSL. For single-strain tests, 200 µl of the Lux-CFP dilution was added with 2 µl of the Rpa-GFP dilution. For all cases, the area of the background mixtures were then divided by the standards to give a population estimate of fluorochrome fluorescence.

These dilutions were then grown for 10 h (non-lysing) or 19 h (with lysis), and the resulting fluorescence time-series traces were used to calculate the estimated populations of the co-cultures. Population estimates in the co-culture mixtures were made in the following way. The GFP fluorescence time-series trace of Rpa-GFP alone was integrated and used as a standard for accumulated fluorescence of a culture with 100% of the Rpa-GFP strain. In the same way, the CFP fluorescence time-series trace of Lux-CFP alone was integrated and used as a standard for accumulated fluorescence of a culture with 100% of the Lux-CFP strain. The integrated GFP and CFP fluorescence curves of the mixtures were then divided by the standards to give a population estimate of Rpa-GFP and Lux-CFP, respectively. For all cases, the area of the background fluorescence was subtracted. Additionally, the CFP fluorescence required extra signal normalization because the Tecan’s GPF sensor reads into the CFP emission profile (but not the other way around).

The equations used to calculate the population estimates, with appropriate filtering and normalization, are as follows:

\[
\text{Population}_{Lux} = \frac{\text{Area}(\text{CFPLux})}{\text{Area}(\text{CFS})} - \frac{\text{Area}(\text{BGFP})}{\text{Area}(\text{BG})}
\]

\[
\text{GFP}_{\text{Crosstalk}} = \frac{\text{Area}(\text{GFPRpa})}{\text{Area}(\text{BG})} - \frac{\text{Area}(\text{CFPLux})}{\text{Area}(\text{CFS})}
\]

\[
\text{GFP}_{\text{Real}} = \text{GFP}_{\text{Crosstalk}} - \frac{\text{Area}(\text{GFPRpa})}{\text{Area}(\text{BG})}
\]

\[
\text{Population}_{Rpa} = \frac{\text{GFP}_{\text{Real}}}{\text{Area}(\text{GFP}_{\text{Real}})} - \frac{\text{Area}(\text{BG})}{\text{Area}(\text{BG})}
\]

\[
\text{Population}_{Lux} = \frac{\text{Area}(\text{CFPLux})}{\text{Area}(\text{CFS})} - \frac{\text{Area}(\text{BGFP})}{\text{Area}(\text{BG})}
\]

\[
\text{GFP}_{\text{Crosstalk}} = \frac{\text{Area}(\text{GFPRpa})}{\text{Area}(\text{BG})} - \frac{\text{Area}(\text{CFPLux})}{\text{Area}(\text{CFS})}
\]

\[
\text{GFP}_{\text{Real}} = \text{GFP}_{\text{Crosstalk}} - \frac{\text{Area}(\text{GFPRpa})}{\text{Area}(\text{BG})}
\]

\[
\text{Population}_{Rpa} = \frac{\text{GFP}_{\text{Real}}}{\text{Area}(\text{GFP}_{\text{Real}})} - \frac{\text{Area}(\text{BG})}{\text{Area}(\text{BG})}
\]

Population\(_{Lux}\) is the population estimate of the Lux-CFP strain in a co-culture. Area(CFPLux) is the area of the CFP fluorescence time-series curve of a given co-culture. Area(BG) is the area of the background CFP fluorescence time-series line. Area(CFPLux) is the average area of the CFP fluorescence time-series curve in the wells with only the Lux-CFP strain. Area(GFPRpa) is the area of the background GFP fluorescence time-series line. Area(BG) is the area of the background GFP fluorescence time-series curve in the wells with only the Lux-CFP strain. Area(GFP_{Real}) is the area of the background GFP fluorescence time-series curve in the wells with only the Rpa-GFP strain. Finally, Population\(_{Rpa}\) is the population estimate of the Lux-CFP strain in a co-culture.

Agent-based modelling. For the agent-based model, to simulate bacterial motion, we adapted the mechanical agent-based model developed in our earlier work\(^6,^7\). Each cell was modelled as a spherocylinder of unit diameter that grows linearly along its axis and divides equally after reaching a critical length \(L = 4\). It can also move along the plane due to forces and torques produced by interactions with other cells. The slightly inelastic cell-cell normal contact forces were computed using the standard spring-dashpot model and the tangential forces were computed as velocity-dependent friction.

To describe the intracellular dynamics of each cell, we adapted the ordinary differential equation model from ref. 16. Specifically, the intracellular dynamics are as follows:

\[
P_L = a_0 + a_1 \left( \frac{H_L H_C}{H_L + H_C} \right) + D_L \left( H_L - H_C \right)
\]

\[
\frac{dH_L}{dt} = -\frac{1}{K_{H_L}} + H_L + D_L \left( H_C - H_L \right)
\]

\[
\frac{dH_C}{dt} = -\frac{1}{K_{H_C}} + H_C + D_L \left( H_L - H_C \right)
\]

\[
\eta \frac{dH_L}{dt} = P_{Lux} - y_L H_L
\]

\[
\frac{d}{dt} = C_P_{Lux} - y_L L
\]

where \(P_{Lux} = H_L, I_L = I_c\), and \(I_c\) are the activity of the LuxI promoter, intracellular AHL, LuxI and lysis protein of the nth cell. \(H_L, I_c\) is the extracellular concentration of AHL at the location of the nth cell. LuxI promoter is induced by AHL \(b(I_L K_t + I)\) is the production term for AHL. \(D_{Lux}(H_L + I - H)\) describes the exchange of intra- and extracellular AHL across the cell membrane. \(P_{Lux} = y_L L\) are the production and degradation terms for LuxI. \(C_P_{Lux}\) and \(y_L L\) are the production and degradation terms for lysis protein.

The extracellular AHL concentration \(H(x, t)\) is governed by the following linear diffusion equation:

\[
\frac{\partial H(x, t)}{\partial t} = D_L \left( H(x, t) - \frac{\partial^2 H(x, t)}{\partial x^2} \right) - \delta_{H_L}(x, t) + D_L \nabla^2 H(x, t)
\]

In the simulation, we use two-dimensional finite difference methods to describe the diffusion of AHL.

We implement the model in trapps with different side lengths \((20, 40 \text{ and } 60 \mu m)\). To simulate the lysis of each cell, we assume that when the concentration of lysis protein \(L \geq 1 \text{ threshold of lysis} \) the cell has a probability of \(P_L = p_2(L_0 - L_0)\) per unit of time to lyse and, once a cell lyse, it is removed from the trap.

We chose model parameters to qualitatively fit the experimental results and the parameters \(H_2, m, b, p_2\) were chosen to account for the differences of experimental measurements and dynamic behaviours between Lux-CFP and Rpa-GFP strains. The parameter values for the Lux-CFP strain are \(a_0 = 0.1 \text{ Lux promoter basal production} = a_1 = 2 \text{ (Lux promoter AHL-induced production)}\), \(b = 1 \text{ (AHL binding affinity to Lux promoter}, m = 4 \text{ (Hill coefficient of AHL-induced production of Lux promoter)}\), \(b = 1.5 \text{ (AHL production rate)}\), \(K_t = 1 \text{ (concentration of LuxI resulting in half maximum production of AHL)}\), \(D_{Lux} = 10 \text{ (diffusion constant of AHL across the cell membrane)}\), \(C_P = 1 \text{ (LuxI copy number)}\), \(y_L = 0.5 \text{ (degradation rate of lysis protein)}\), \(p_1 = 0.1 \text{ (diffusion rate of extracellular AHL)}\), \(p_2 = 0.3 \text{ (probability of lysis)}\) and \(L_0 = 1.6 \text{ (threshold of lysis protein for lysis)}\). To simulate the constant-lysis Rpa-GFP strain, these parameters have different values: \(H_2 = 0.2, m = 1 \text{, } b = 0.6, p_1 = 0.03\). Also, the growth rate of the Rpa-GFP strain is 10% larger than that of the Lux-CFP strain.

Deterministic modelling

Single lysis oscillator strain. We describe the population-level mechanisms that lead to oscillations in population size as observed with the synchronized lysis circuit. To gain an intuitive understanding, we used a reduced model that aims to reproduce the observed population level behaviour using only the fundamental ingredients of the circuit: autocatalytic production of the QS agent and QS agent-induced lysis of cells. The basic equations for a single strain equipped with the lysis circuit are as follows (see Supplementary Fig. 5 for model traces):
where \( \alpha \) is the concentration of \( q \) that results in the half-maximum death rate (and auto-catalysed production of \( q \)) and \( m \) is the Hill coefficient.

A linear stability analysis shows that the system (1, 2) has a stable fixed point when

\[
m - 1 \frac{a}{\gamma} y < 1 + \frac{a}{\gamma} y \frac{\alpha}{\alpha_a}
\]

The border of this stability region corresponds to the onset of oscillations. Basal parameters are, unless otherwise mentioned, \( a = 1, y = 4, \alpha_a = 0.4, \gamma = 1, \gamma_r = 1, \alpha = 2 \). These parameters lead to oscillations according to equation (4). All simulations are carried out using the Runge–Kutta–Fehlberg (RKF45) method. An example trajectory is depicted in Supplementary Fig. 5.

Although we do not explicitly model individual proteins or enzymes, we can gain an understanding for the influence of LuxI degradation by ClpXP with model (1, 2) using the following logic. When there is very little LuxI (that is, the positive feedback loop has not been activated), fast degradation by ClpXP will have a strong influence on the steady-state level of LuxI. LuxI with a strong degradation tag will experience fast degradation by ClpXP, leading to a low basal production rate of QS agent \( \alpha \), whereas LuxI with a weak degradation tag will have a higher steady-state level and therefore a higher basal production rate \( \alpha \). In contrast, once the positive feedback has been activated, the concentration of LuxI (and consequently parameter \( \alpha \) of the model) has a much weaker dependence on its degradation tag because an abundance of LuxI produced from a fully activated promoter saturates the limited enzymatic processing capacity of ClpXP and therefore the level of LuxI will be determined mainly by dilution due to cell growth. As seen from equation (4), decreasing \( \alpha \) by a larger factor than \( \alpha \) generally brings the system closer to oscillations, which is consistent with the requirement for a strong degradation tag for sustained oscillations demonstrated in Fig. 2. In summary, we model stronger (weaker) enzymatic degradation of LuxI by a lower (higher) value of \( \alpha \).

**Microfluidic traps and multiple strains.** A microfluidic trap is clearly a finite environment, but because nutrients are constantly replenished by diffusion from faster growing cells, the trap can (as often assumed in other scenarios with finite carrying capacities) be unrealistic of the description of the population dynamics. Instead, we assume that growth is unaffected as long as the population density is below the carrying capacity \( c \) of the trap. We then cap the cell density at \( c \), corresponding to any extra cells being washed away by the flow in the main channel (‘plug flow’). Notably, this results in a net decrease of \( c \) after every time step of the simulation if it exceeds \( c \). In all our simulations \( c = 1 \). Supplementary Fig. 5 shows that the system with standard parameters lyses just before it reaches the carrying capacity of the trap, so it is truly self-limiting.

For simulations of multiple strains, we simulate two copies of system (1, 2) with variables \( n_1, q_1 \) and \( n_2, q_2 \). Again, we let the system evolve freely as long as \( n_1 + n_2 < c \). If \( n_1 + n_2 > c \) after any one time step, we set \( n_1 = n_1^* \) and \( n_2 = n_2^* \),

\[
n_1 = n_1^* + \frac{n_2^* - n_1^*}{\gamma} c \quad n_2 = n_2^* + \frac{n_1^* - n_2^*}{\gamma} c \quad \text{if} \quad n_1 + n_2 > c
\]

where \( n_1^* \) and \( n_2^* \) correspond to the population densities before the reset. More specifically, this way of limiting the total population density to the carrying capacity \( c \) corresponds to a well-mixed environment, such that the relative population densities of the two strains remain unchanged upon lysis.

Consequently, two oscillating strains in one trap that use completely different parameters are, unless otherwise mentioned, \( a = 1, y = 4, \alpha_a = 0.4, \gamma = 1, \gamma_r = 1, \alpha = 2 \). These parameters lead to oscillations according to equation (4) and are assumed in other scenarios with finite carrying capacities.

Data availability. The data that support the findings of this study are available from the corresponding author upon request.

**Code availability.** The modelling code for the agent-based as well as deterministic numerical simulations is available from the corresponding author upon request.

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Author contributions
All authors contributed extensively to the work presented in this paper.

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Competing interests
The authors declare no competing financial interests.