A synchronized quorum of genetic clocks

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The engineering of genetic circuits with predictive functionality in living cells represents a defining focus of the expanding field of synthetic biology. This focus was elegantly set in motion a decade ago with the design and construction of a genetic toggle switch and an oscillator, with subsequent highlights that have included circuits capable of pattern generation, noise shaping, edge detection and event counting. Here we describe an engineered gene network with global intercellular coupling that is capable of generating synchronized oscillations in a growing population of cells. Using microfluidic devices tailored for cellular populations at differing length scales, we investigate the collective synchronization properties along with spatiotemporal waves occurring at millimetre scales. We use computational modelling to describe quantitatively the observed dependence of the period and amplitude of the bulk oscillations on the flow rate. The synchronized genetic clock sets the stage for the use of microbes in the creation of a macroscopic biosensor with an oscillatory output. Furthermore, it provides a specific model system for the generation of a mechanistic description of emergent coordinated behaviour at the colony level.

Synchronized clocks are of fundamental importance in the coordination of rhythmic behaviour among individual elements in a community or a large complex system. In physics and engineering, the Huygens paradigm of coupled pendulum clocks1–6 has permeated diverse areas from the development of arrays of lasers6 and superconducting junctions7 to Global Positioning System (GPS)7 and distributed sensor networks8. In biology, a vast range of intercellular coupling mechanisms lead to synchronized oscillators that govern fundamental physiological processes, such as somitogenesis, cardiac function, respiration, insulin secretion and circadian rhythms7–9. Typically, synchronization helps stabilize a desired behaviour arising from a network of intrinsically noisy and unreliable elements. Sometimes, however, the synchronization of oscillations can lead to severe malfunction of a biological system, as in epileptic seizures9,10.

There is considerable interest in the use of synthetic biology to recreate complex cellular behaviour from the underlying biochemical reactions that govern gene regulation and signalling. Synthetic biology can be broadly parsed into efforts aimed at the large-scale synthesis of DNA and the forward engineering of genetic circuits from known biological components. In the area of DNA synthesis, pathways have been perturbed and replaced11 in an effort to understand the network motifs and transcriptional regulatory mechanisms that control cellular processes and elicit phenotypic responses12. On a larger scale, progress has been made towards the creation of entire genomes, providing new insights into what constitutes the minimal set of genes required for microbial life13.

The genetic circuits approach to synthetic biology involves the forward engineering of relatively small gene networks using computational modelling14,15. Here, the original toggle switch16 and oscillator17 have inspired the design and construction of circuits capable of controlling cellular population growth18, generating specific patterns19, triggering biofilm development20, shaping intracellular noise21, detecting edges in an image22 and counting discrete cellular events23. In the context of rhythmic behaviour, there have been recent successes in the construction of intracellular oscillators that mimic naturally occurring clocks24–26. As well as their potential as biological sensors, these clock networks have led to insights about the functionality of circadian networks27,28. A unifying theme for most of the genetic circuit studies is a particular focus on dynamical behaviour. Thus, the circuits are constructed and monitored in single cells, typically with fluorescent reporters, and new measurement technologies are often developed in parallel29. Furthermore, because nonlinearities and stochasticity arise naturally, tools from the fields of nonlinear dynamics and statistical physics are extremely useful both in the generation of design specifications and for careful comparison between experiment and computational model.

Synchronized genetic oscillators

The synchronized oscillator design (Fig. 1a) is based on elements of the quorum sensing machineries in Vibrio fischeri and Bacillus Thurigensis. We placed the lux operon (from V. fischeri), aiiA (from B. Thurigensis) and yemGFP genes under the control of three identical copies of the luxO promoter. The LuxI synthase enzymatically produces an acyl-homoserine lactone (AHL), which is a small molecule that can diffuse across the cell membrane and mediates intercellular coupling. It binds intracellularly to the constitutively produced LuxR, and the LuxR–AHL complex is a transcriptional activator for the luxO promoter30. AiiA negatively regulates the promoter by catalysing the degradation of AHL31. This network architecture, whereby an activator activates its own protease or repressor, is similar to the motif used in other synthetic oscillator designs32–34 and forms the core regulatory module for many circadian clock networks35,36,37. Furthermore, theoretical work has shown how the introduction of an autoinducer in similar designs can potentially lead to synchronized oscillations over a population of cells38,39.

Most quorum sensing systems require a critical cell density for generation of coordinated behaviour40. We modified the local cell density of the synchronized oscillator cells (denoted TDQS1) through the use of microfluidic devices41,42 of differing geometries. The device used for monitoring the bulk oscillations consists of a main nutrient-delivery channel that feeds a rectangular trapping chamber (Fig. 1b). Once seeded, a monolayer of Escherichia coli cells grow in the chamber and cells are eventually pushed into the channel where they then flow to the waste port. This device allows for a constant supply of nutrients

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or inducers and the maintenance of an exponentially growing colony of cells for more than 4 days. We found that chamber sizes of 100 $\times$ (80–100) $\mu$m$^2$ were ideal for monitoring the intercellular oscillator, as they allowed for sufficient nutrient distribution and optimal cell and AHL densities. In the context of the design parameters, the flow rate can be modulated to change the local concentration of AHL. Furthermore, the device can be modified to permit the observation of spatial waves over longer length scales.

After an initial transient period, the TDQS1 cells exhibit stable synchronized oscillations that are easily discernible at the colony level (Fig. 1c, d and Supplementary Movies 1–2). The dynamics of the oscillations can be understood as follows. Because AHL is swept away by the fluid flow and is degraded by AiiA internally, a small colony of individual cells cannot produce enough inducer to activate expression from the luxI promoter. However, once the population reaches a critical density, there is a ‘burst’ of transcription of the luxI promoters, resulting in increased levels of LuxI, AiiA and green fluorescent protein (GFP). As AiiA accumulates, it begins to degrade AHL, and after a sufficient time, the promoters return to their inactivated state. The production of AiiA is then attenuated, which permits another round of AHL accumulation and another burst of the promoters.

To determine how the effective AHL dissipation rate affects the period of the oscillations, we conducted a series of experiments at various channel flow rates. At high flow rate, the oscillations stabilize after an initial transient and exhibit a mean period of 90 $\pm$ 6 min and mean amplitude of 54 $\pm$ 6 GFP arbitrary units (Fig. 2a and Supplementary Movie 2). At low flow rate, we observed a period of 55 $\pm$ 6 min and amplitude of 30 $\pm$ 9 GFP arbitrary units. Notably, the waveforms have differing shape, with the slower oscillator reaching a trough near zero after activation, and the faster oscillator decaying to levels above the original baseline (Fig. 2b). We swept the flow rate from 180 to 296 $\mu$m s$^{-1}$ and observed an increasing oscillatory period from 52–90 min (Fig. 2c). Moreover, we found the amplitude to be proportional to the period of the oscillations (Fig. 2d), which is consistent with ‘degrade-and-fire’ oscillations observed in a previously reported intracellular oscillator.

**Spatiotemporal dynamics**

In experiments conducted at low flow rate, we observed the spatial propagation of the fluorescence signal across the 100-$\mu$m chamber, controlled to change the effective degradation rate of AHL. c, Bulk fluorescence as a function of time for a typical experiment in the microfluidic device. The red circles correspond to the image slices in d. a.u., arbitrary units. d, Fluorescence slices of a typical experimental run demonstrate synchronization of oscillations in a population of E. coli residing in the microfluidic device (Supplementary Movie 1). Inset in the first snapshot is a $\times$100 magnification of cells.

To investigate these spatiotemporal dynamics in more detail, we redesigned the microfluidic chip with an extended 2-mm trapping chamber (Supplementary Information). Snapshots of a typical experimental run are presented in Fig. 3a (Supplementary Movies 3 and 4). A few isolated colonies begin to grow and subsequently merge into a large monolayer that fills the chamber (Fig. 3a, 66 min). At 100 min, there is a localized burst of fluorescence that propagates to the left and right in subsequent frames (Fig. 3a, 100–118 min). A second burst occurs near the original location and begins to propagate to the left and right as before.

To illustrate the spatiotemporal information contained in an entire 460-min image sequence, we plot the fluorescence intensity as a function of time and distance along the chamber (Fig. 3b). Note

![Figure 1](https://example.com/figure1)

**Figure 1 | Synchronized genetic clocks.** a, Network diagram. The luxI promoter drives production of the luxI, aiiA and yemGFP genes in three identical transcriptional modules. LuxI enzymatically produces a small molecule AHL, which can diffuse outside of the cell membrane and into neighbouring cells, activating the luxI promoter. AiiA negatively regulates the circuit by acting as an effective protease for AHL. b, Microfluidic device used for maintaining E. coli at a constant density. The main channel supplies media to cells in the trapping chamber, and the flow rate can be externally

![Figure 2](https://example.com/figure2)

**Figure 2 | Dynamics of the synchronized oscillator under several microfluidic flow conditions.** (See also Supplementary Movies 1 and 2.) a, At around 90 min, cells begin to oscillate synchronously after reaching a critical density in the trap. b, The period and amplitude increase for higher flow rates. Magenta curve is at low velocity (240 $\mu$m min$^{-1}$), blue is at higher velocity (280 $\mu$m min$^{-1}$). c, Period as a function of velocity in the main channel showing tunability of period between 55–90 min. d, Period versus amplitude for all experiments. Magenta circles (c, d) are data from 84 and 90 $\mu$m traps, blue crosses are 100 $\mu$m traps. Error bars in c and d indicate $\pm$1 s.d. for a single channel, averaged over 10–50 peaks; each data point represents a different run.
During the first 100 min, there is no activity and the space–time plot is blue, indicating no fluorescence. Then at 100 min, there is an orange spot at around 1,350 μm, corresponding to the burst in Fig. 3a. In the space–time plot, propagation of a wave to the left and right appears as a green–yellow concave line. The larger slope to the left of the burst origin indicates that the leftward moving wave is travelling slower (~25 μm min⁻¹) than the rightward wave (~35 μm min⁻¹). Subsequent waves originating from a nearby location arise as further orange–yellow intensity lines. These intensity lines indicate ‘annihilation events’, where leftward moving and rightward moving waves collide and annihilate each other. Although these events are prominent in the movies (Supplementary Movies 3 and 4) they appear subtly in the space–time plot at locations where positive and negative slopes meet (300–400 μm in second intensity line and on). As the travelling wave gets further from a burst location it breaks off into a packet (170 min) that travels leftward at 12.5 μm min⁻¹ initially, and slows to 8.5 μm min⁻¹ towards the end of the trap where the cell density is lower (between 118–200 min). The corresponding cell-density space–time plot shows that a higher density of cells is first reached at the centre of the colony and is minimal towards the left-moving edge (Supplementary Fig. 3 and Supplementary Movie 3). As a result, the critical cell and AHL densities for wave propagation are reached at different times and spatial locations.

We also investigated how the intercellular oscillator behaves in a three-dimensional colony growing in a 400 × 1,000 × 4.0 μm³ microfluidic chamber (Fig. 3c, d and Supplementary Movie 5). In this device, the colony grows radially over the course of 180 min without fluorescing until it reaches a size of approximately 100 μm. At this time, a large fluorescence burst originates from the centre of the colony, with a bright band near the centre (Fig. 3c, 228 min). During this first burst (273 min), the bright band shows that cells at an intermediate cell density have a larger amplitude and longer period than cells near the front or in the interior.

Fluorescence of cells along the centre of the trap as a function of time.

Figure 3 | Spatiotemporal dynamics of the synchronized oscillators. a, Snapshots of the GFP fluorescence superimposed over brightfield images of a densely packed monolayer of E. coli cells are shown at different times after loading (Supplementary Movies 3 and 4). Travelling waves emerge spontaneously in the middle of the colony and propagate outwards with a speed of ~8–35 μm min⁻¹. At later times waves partially lose coherence owing to inhomogeneity in cell population and intrinsic instability of wave propagation. b, Corresponding space–time diagram showing the correspondence of this space–time plot to the images in Fig. 3a. Bursts of fluorescence begin when the growing colony reaches a critical size of about 100 μm. These bursts are primarily localized at the periphery of the growing colony. c, Snapshots of the GFP fluorescence superimposed over the brightfield images of a three-dimensional growing colony of E. coli cells at different times after loading (Supplementary Movie 5). Bursts of fluorescence begin when the growing colony reaches a critical size of about 100 μm. These bursts are primarily localized at the periphery of the growing colony. d, Corresponding space–time diagram showing fluorescence of cells along a horizontal line through the centre of the growing colony.

Quantitative modelling

To describe quantitatively the mechanisms driving bulk synchronization and wave propagation, we developed a computational model using delayed differential equations for protein and AHL concentrations (Supplementary Information). Although conceptually the nature of oscillations is reminiscent of the degrade-and-fire oscillations observed in a dual delayed feedback circuit, an important difference is the coupling among genetic clocks in different cells through extracellular AHL. The modelling of this coupling, and the related cell-density dependence, allowed us to explain most of the non-trivial phenomenology of the spatiotemporal quorum clock dynamics.

A broad range of model parameters lead to oscillations (Fig. 4a–d), although there is a distinct absence of oscillations at small and large cell densities for low to medium flow values (Fig. 4c). The qualitative nature of the oscillations can be explained using Fig. 4a. Each period begins with the latent accumulation of both AiiA and LuxI, which after a delay burst rapidly to high values. That burst suppresses AHL and further production of AiiA and LuxI. Both of these proteins then decay enzymatically, after which the process repeats. As expected, the period of the oscillations is roughly proportional to the enzymatic protein decay time. The period grows with the external AHL flow rate (effective degradation) and the amplitude of the oscillations, in good agreement with the experiments (compare Fig. 4b with Fig. 3c, d).

We modelled the collective spatiotemporal dynamics of the clocks by generalizing the bulk model to include the coupling of individual oscillators through extracellular AHL. The model consists of a one-dimensional array of ‘cells’, each of which is described by the same set of delay-differential equations coupled to a common, spatially
The results in densities, and period increases with density and AHL decay rate values of other, and are anti-phase with the concentrations of external and internal (green line) and external AHL (red line). LuxI and AiiA closely track each other, and are in agreement with our experimental findings (compare Figs 3b and 4e). The velocity of the front propagating through the cell membrane and dilution. A small AHL perturbation in the middle of the array initiates waves of LuxI concentration (Fig. 4e), in excellent agreement with the experimental data. Furthermore, cell density has an important role in the growing colony of cells. This phenomenology is also in excellent agreement with our experimental findings (compare Figs 4d and 3d).

Perspective and outlook

In the mid-seventeenth century, Chirstiaan Huygens serendipitously observed that two pendulum clocks oscillated in synchrony when mounted to a common support beam. Although observations of synchronization in nature surely predate the age of enlightenment, Huygens is credited as the first to systematically characterize the synchronization of oscillators in terms of a known coupling mechanism (which, in the case of the pendula, he deduced as vibrations in the common support). We have shown how quorum sensing can be used to couple genetic clocks, leading to synchronized oscillations at the colony level. Given the single-cell variability and intrinsic stochasticity of most synthetic gene networks, the use of quorum sensing is a promising approach to increasing the sensitivity and robustness of the dynamic response to external signals. Along these lines, our results set the stage for the design of networks that can function as spatially distributed sensors or synthetic machinery for coupling complex dynamical processes across a multicellular population.

METHODS SUMMARY

Strains, growth conditions. Three identical transcriptional cassettes for luxI, aiiA and yemGFP were constructed by replacing the promoter of a modular pZ-plasmid (with yemGFP) with the lux operon from the native V. fischeri operon (luxR up to lux stop codon)\(^1\). luxI and aiiA\(^*\) genes were cloned in place of yemGFP, and a degradation tag was added to the carboxy-terminal of each. A previously used MG1655 strain of E. coli\(^1\) was transformed with plasmids pTD103luxI/GFP(colE1,Kan) and pTD103aiiA(p15A,Amp) to create strain TDQS1 (Supplementary Information).

Each experiment started with a 1:1,000 dilution of overnight culture grown in 50 ml LB (10 g l\(^{-1}\) NaCl) with antibiotics 100 μg ml\(^{-1}\) ampicillin (Amp) and 50 μg ml\(^{-1}\) kanamycin (Kan) for approximately 2 h. Cells reached an\(\Delta_{600\text{nm}}\) of 0.05–0.1, and were spun down and concentrated in 5 ml of fresh media with surfactant concentration of 0.075% Tween20 (Sigma-Aldrich) before loading in a device.

Microfluidics and microscopy. Images were acquired using an epifluorescent inverted microscope (TE2000-U, Nikon Instruments Inc.), and chip temperatures were maintained at 37 °C with a plexiglass incubation chamber encompassing the entire microscope. Phase-contrast and fluorescent images were taken at ≥20 or ≥60 every 2–5 min, and focus was maintained automatically using Nikon Elements software.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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