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Communication and Computation by Bacteria Compartmentalized within Microemulsion Droplets

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Supporting Information

ABSTRACT: Amphiphilic inducer molecules such as *N*-acyl-L-homoserine lactones (AHLs) or isopropyl-*β*-D-thiogalactopyranoside (IPTG) can be utilized for the implementation of an artificial communication system between groups of *E. coli* bacteria encapsulated within water-in-oil microemulsion droplets. Using spatially extended arrays of microdroplets, we study the diffusion of both AHL and IPTG from inducer-filled reservoirs into bacteria-containing droplets, and also from droplets with AHL producing sender bacteria into neighboring droplets containing receiver cells. Computational modeling of gene expression dynamics within the droplets suggests a strongly reduced effective diffusion coefficient of the inducers, which markedly affects the spatial communication pattern in the neighborhood of the senders. Engineered bacteria that integrate AHL and IPTG signals with a synthetic AND gate gene circuit are shown to respond only in the presence of both types of sender droplets, which demonstrates the potential of the system for genetically programmed pattern formation and distributed computing.

Quorum sensing (QS) is a mode of chemical communication between bacteria mediated by diffusible inducer molecules called autoinducers (AIs). In a typical QS system, AIs are produced by an AI synthase, whose production is itself controlled by the presence of AI. Due to the membrane-penetrating nature of the AIs, AI-inducible genes may also be influenced in neighboring bacteria, which facilitates the induction of genes in a cell-density dependent manner, hence the name "quorum sensing".[1] QS has been identified both in gram negative as well as in gram positive bacteria, with different classes of AIs.[2] Chemically, the AI-1 class AIs are *N*-acyl-L-homoserine lactones (AHLs), which are found in gram negative bacteria. Specifically, the AI-1 of the bacterium *Aliivibrio fischeri*[3] used in the present work—regulating the LuxR-LuxI system—is the amphiphilic molecule *N*-(3-oxohexanoyl)-L-homoserine lactone (3OC6HSL).

In the context of synthetic biology, bacterial QS systems have been frequently utilized as a means of communication between engineered bacteria. In a seminal work, Weiss and Knight[4] artificially separated the QS system of *A. fischeri* into "sender" and "receiver" parts, and were therefore able to realize the first synthetic bacterial communication system. Based on this sender-receiver system, various gene circuits were engineered, such as a population control system,[5] pattern-forming systems,[6] or distributed bacterial computing.[7] Artificial microenvironments and microfluidics[8] have been previously used to investigate spatial aspects of cell-to-cell communication via QS. For instance, QS between groups of bacteria was studied using inkjet printing,[9] fiberoptic microarrays[10] or by microfluidic confinement within small aqueous droplets.[11] Recently, also signaling between bacteria confined in an emulsion droplet to a neighboring compartment within a microfluidic double droplet trap was shown,[12] a mechanism that is also used in our work.

Here, we utilize a genetically engineered QS-based sender-receiver system, but also the conventional inducer/repressor module isopropyl-*β*-D-thiogalactopyranoside (IPTG)/LacI to implement a spatially extended communication system between *E. coli* bacteria encapsulated within large populations of water-in-oil emulsion droplets (cf. Figures S1 and S2). We find that both AHL (3OC6HSL) and IPTG partly dissolve in the oil phase (Figures S5 and S6). The resulting slow diffusion of the inducers from compartment to compartment establishes two chemical communication channels between the droplets, which can be directly demonstrated using computational bacteria that process AHL and IPTG signals as inputs of a simple genetic AND gate. Integration of several slowly diffusing signals within a droplet population using synthetic gene regulatory circuits is expected to enable programmable pattern formation[6a] and distributed computing[7,13] on a shorter length scale than in aqueous medium.[14]

We first investigated the influence of inducer-filled "reservoir" droplets without bacteria on gene expression in "receiver cells" in neighboring droplets (Figure 1A). Emulsion droplets were formed using a microfluidic flow-focusing device made of PDMS, in which an aqueous phase was mixed with fluorocarbon oil containing a nonionic, biocompatible surfactant.[15] A schematic representation of the receiver genetic circuit is shown in Figure 1b. Receiver bacteria constitutively expressed the *A. fischeri* QS regulatory protein LuxR, which acts as an activator of gene expression upon binding to AHL. As a read-out for the presence of AHL, the expression of green fluorescent protein (GFP) was put under the control of the AHL-inducible promoter pLuxR. Cell densities were chosen sufficiently low to ensure that either no or only few bacteria were present within each droplet initially.

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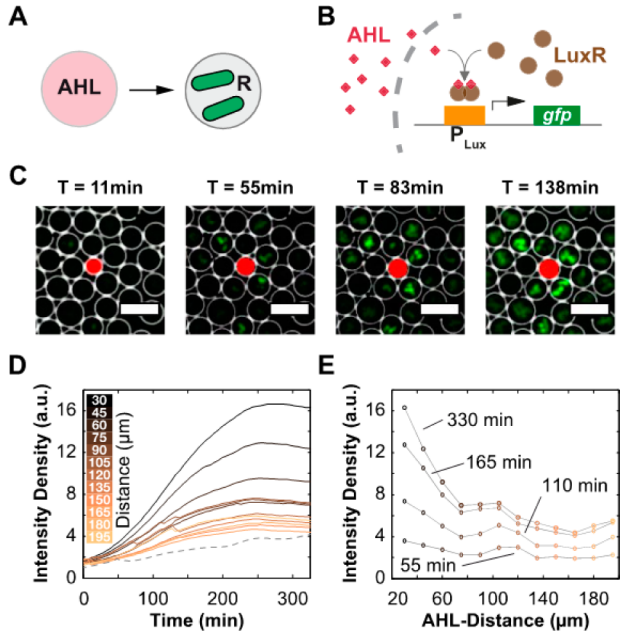

Figure 1. (A) Receiver bacteria in the presence of AHL reservoir droplets. (B) Receiver gene circuit. AHL enters a receiver cell through the cell membrane (dashed line) and binds to constitutively expressed LuxR. LuxR:AHL dimers activate expression of GFP. (C) Fluorescence microscopy time series of receiver cells close to an AHL reservoir droplet (red). White circles are derived from bright field (BF) images and represent droplet surfaces. AHL diffuses from reservoir to receiver droplets and induces gene expression in a distant dependent manner (scale bar, 50 μm; dark droplets close to reservoirs do not contain cells, cf. Figure S2 for higher magnification and BF). (D) Evolution of the average fluorescence intensity of droplets containing receiver cells for different distances from the next nearest reservoir droplet. An appropriately scaled fluorescence time trace from a control experiment with uninduced receiver cells is included for comparison (dashed line). (E) Intensity profiles for different times obtained from the traces in (D).

In Figure 1C, images extracted from a fluorescence microscopy time-lapse movie of a reservoir droplet surrounded by receiver cell droplets are shown, which confirm that gene expression is first induced in droplets adjacent to the reservoirs and starts later in receiver droplets further apart. Gene expression experiments in bulk showed that in the presence of AHL pLuxR-controlled GFP production follows an activation function $\sim 1/(1 + K_{AHL}^n/[AHL]^n)$, with a threshold concentration of $K_{AHL} \approx 15$ nM, and a Hill exponent of $n = 1.6$ (Figure S3). The inducer concentration in the reservoir droplets was therefore chosen to be [AHL] = 200 nM, i.e., more than 10 times the induction threshold.

In order to study the spatiotemporal dynamics of gene expression, in Figure 1D the change of fluorescence collected from 1405 bacteria-filled droplets grouped according to their distance to the next nearest AHL reservoir droplets (in total 91) is shown as a function of time (cf. Figure S1 for image processing). As the droplets roughly arrange in a hexagonally closed packed lattice, the mean distance between reservoir droplets is estimated to be $\approx$66 μm. Thus, the curves only approximately reflect the true distance dependence of gene expression that would be caused by an isolated reservoir droplet. Fluorescence profiles for different distances from reservoir droplets 55, 110, 165, and 330 min after initiation of the experiment are shown in Figure 1E. A clear response of receiver bacteria is visible up to distances of $\sim$100 μm from the reservoir droplets. Qualitatively, inducers diffuse away from the source droplets and induce GFP expression in neighboring receiver droplets. Due to depletion of the reservoirs, decay of inducers and gene products and also due to limited growth of the bacteria in the droplets, gene activation is only transient, leading to the particular shape of the response curves displayed in Figure 1D,E. Figure 1D can be understood more quantitatively in terms of a simple reaction–diffusion (RD) model (SI section 4.7, Figures S8 and S9), yet only with the assumption of a strongly reduced effective diffusivity of AHL on the order of $D_{eff} \approx 1$ μm^2/s as compared to the bulk case, for which diffusivities in the range of $D_{bulk} \approx 100-1000$ μm^2/s are typically assumed.[9,13a,14,16] As discussed in SI section 4.7, this may be explained by varying diffusion coefficients in the different phases, the permeability of the interfaces, and also by geometrical effects.

In order to address the question, whether communication takes place mainly through the interface formed by surfactants between droplets in direct physical contact or via free diffusion through the oil phase, we performed a series of control experiments (Figure S5). We found that 3OC6HSL partly dissolves in the oil phase, which is in accordance with its slight hydrophobicity characterized by an octanol/water partitioning coefficient (log P) in the range 0.2–2,[17] and we also found gene induction in isolated receiver droplets not in physical contact with senders. It is thus conceivable that transport occurs both directly through the interface between touching droplets and via the oil phase.

We reasoned that a droplet-to-droplet induction might also be possible with a conventional inducer such as IPTG, in particular as its chemical structure also suggests an amphiphilic nature[18] (for the IPTG activation function, cf. Figure S4 and SI section 4.5). Experiments equivalent to those with AHL were performed with IPTG-containing droplets and droplets with corresponding receiver bacteria, which contained a gene for a red fluorescent protein (RFP) under the control of a Lac promoter. Indeed, expression of RFP was observed in these bacteria only in the presence of reservoir droplets (see Figure S7). We found that also IPTG partitions into the oil phase (Figure S6), and the dynamics of gene expression was again consistent with a strongly reduced effective diffusion coefficient for the inducer (Figure S10).

In order to demonstrate spatially extended chemical communication between bacteria within droplet arrays, we also performed experiments, in which we exchanged inducer-filled reservoir droplets by droplets containing bacteria expressing the AHL synthase LuxI ("sender cells", Figure 2A). The genetic module responsible for AHL synthesis is shown in Figure 2B. As expected, the fluorescence in receiver cells increases as sender cells synthesize AHL, which is transduced through the emulsion (Figure 2C). Analysis of signals recorded from 1679 receiver droplets mixed with 13 sender droplets again reveals distance-dependent GFP expression levels of receiver cells as shown in Figure 2D. This gene activation pattern is very well reproduced by our RD model when reservoirs with a finite supply are exchanged for permanently producing inducer sources (Figure S11).

With two diffusible inducer molecules available, we investigated an emulsion mixture of droplets containing engineered bacteria, which responded to the simultaneous presence of IPTG and AHL as inputs (Figure 3A). To this end, a genetic AND gate was constructed, in which IPTG induced

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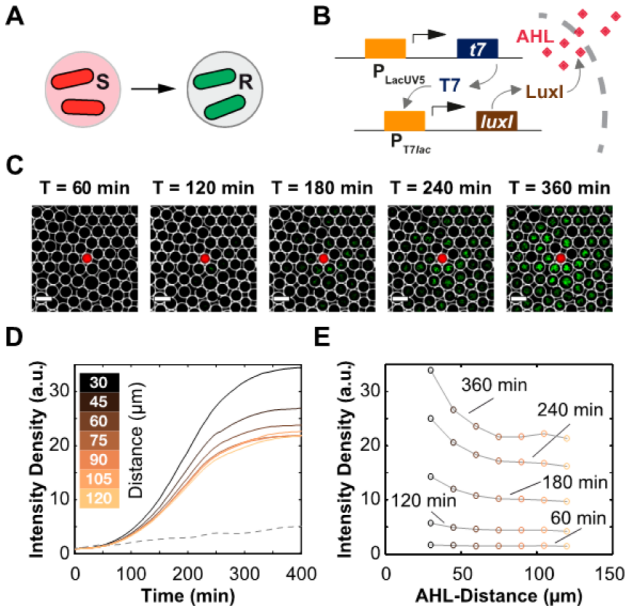

Figure 2. (A) Receiver bacteria in the presence of droplets containing sender cells. (B) Sender gene circuit. A T7 RNA polymerase expressed from an IPTG inducible promoter drives the expression of AHL synthase LuxI. AHL diffuses through the cell membrane (dashed line) into the extracellular medium. (C) Fluorescence microscopy time series recorded from receiver cells in the proximity of a sender droplet (red). AHL spreads by diffusion and activates GFP expression in receiver cells (green). Scale bar, 25 μm. (D) Evolution of the average fluorescence intensity of droplets containing receiver cells for various distances from the next nearest sender-containing droplet. A fluorescence time trace from a control experiment with uninduced receivers cells is also shown (dashed line). (E) Intensity profile at different times corresponding to the traces shown in (D).

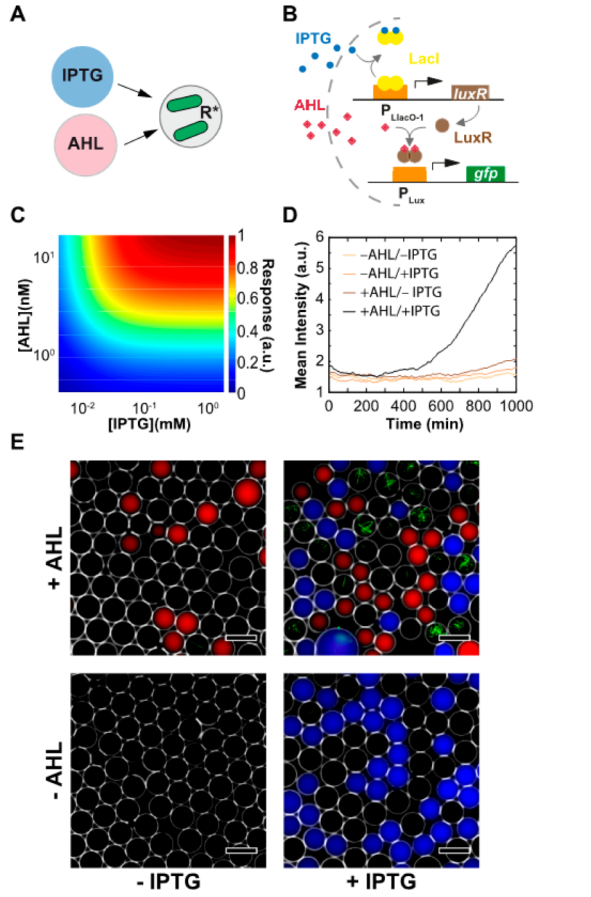

Figure 3. (A) Receiver bacteria containing a genetic AND gate. AHL and IPTG diffuse from reservoir droplets to a receiver droplet with engineered bacteria containing a genetic AND gate. (B) AND-gate gene circuit in the presence of both input molecules. IPTG enters the bacterial cell, binds to the Lac repressor LacI and thus induces expression of LuxR. AHL then binds to LuxR and thus activates expression of GFP. (C) Response of the genetic AND-gate to varying input molecule concentrations. The diagram shows the fit of a two-dimensional input function to values determined in bulk measurements (SI section 4.5). (D) Fluorescence time traces of droplet populations containing computational receiver bacteria in the presence (+) or absence (−) of inducer-filled reservoir droplets (for single droplet traces see Figure S12). (E) Fluorescence microscopy images arranged in a truth table (BF images in Figure S13). AND-gate bacteria express GFP when both AHL (red) and IPTG (blue) reservoir droplets are present. Scale bar, 50 μm.

expression of LuxR, while AHL activated GFP expression via binding to LuxR (Figure 3B). We first characterized the operation of the AND gate circuit in bulk experiments (SI section 4.5), from which the dose−response function depicted in Figure 3C was extracted. As designed, GFP expression was high only in the presence of both inducers. We then studied the response of droplets containing AND-gate bacteria in the presence of reservoir droplets with low inducer concentrations ([AHL] = 20 nM, [IPTG] = 200 μM). In Figure 3D, the corresponding population mean of single droplet fluorescence time traces are shown. As desired, encapsulated AND gate bacteria express GFP only when both AHL and IPTG filled reservoir droplets are present, whereas expression remains low in the absence of one or both inducers. Fluorescence microscopy images of AND-gate bacteria droplets in the presence of either no, one, or both types of inducer droplets taken 18 h after initiation of the experiment are shown in Figure 3E. As expected, green fluorescence is high only when both inducer filled droplets are present. For reservoir droplets with higher inducer concentrations—and thus larger "diffusion range"—also spatial effects can be observed. The AND gate response for this case as a function of distance to the nearest AHL and IPTG reservoirs is shown in Figure S14.

We have shown that amphiphilic inducer molecules such as the QS signal 3OC6HSL or the conventional inducer IPTG can establish chemical communication between chemical reservoirs and small groups of bacteria encapsulated within water-in-oil microemulsion droplets, and this presumably holds true also for other amphiphilic compounds, e.g., antibiotics. Using engineered computational bacteria, several of such signals sent out from distinct reservoir droplets can be integrated within receiver droplets in a context-dependent manner. As natural QS occurs in complex environments such as biofilms,[1c,16] emulsion systems may actually be used as models for the study of bacterial communication in heterogeneous media. Apart from this, a small, potentially tunable diffusivity for bacterial communication could be of considerable interest for applications in synthetic biology. Several studies have explored genetically programmed structure formation in the past,[6a,13a] resulting in patterns on a millimeter length scale. Tuning of the diffusion coefficient to smaller values would reduce the patterning length scale by a factor $(D_0/D_{eff})^{1/2}$, which is of order ∼10 in our case.

An additional interesting feature of a droplet-based bacterial communication system is the fact that communication takes

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place between small and spatially separated groups of bacteria. In principle, different types (even species) of bacteria with potentially different environmental requirements could be prepared in different droplets, and their interactions studied without mixing of the bacteria themselves.

Another aspect of compartmentalization is the fact that chemical signals are sent out or received by small groups of bacteria, which is expected to average out fluctuations caused by single cell variability.[19] Previous studies on distributed computing based on spatially separated, communicating microcolonies[7,13b] have emphasized the fact that such averaging makes bacterial computing more robust than in alternative concepts based on intracellular (single cell) computing. A distributed bacterial computer implemented in microemulsions potentially could be faster and operate with reduced space requirements.

■ ASSOCIATED CONTENT

Supporting Information

Materials, methods, additional data, and modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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