

Supplementary Information:

Chemical communication between bacteria and cell-free gene expression systems within linear chains of emulsion droplets

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Supplementary References

1. Bulk characterization of synthetic gene circuits

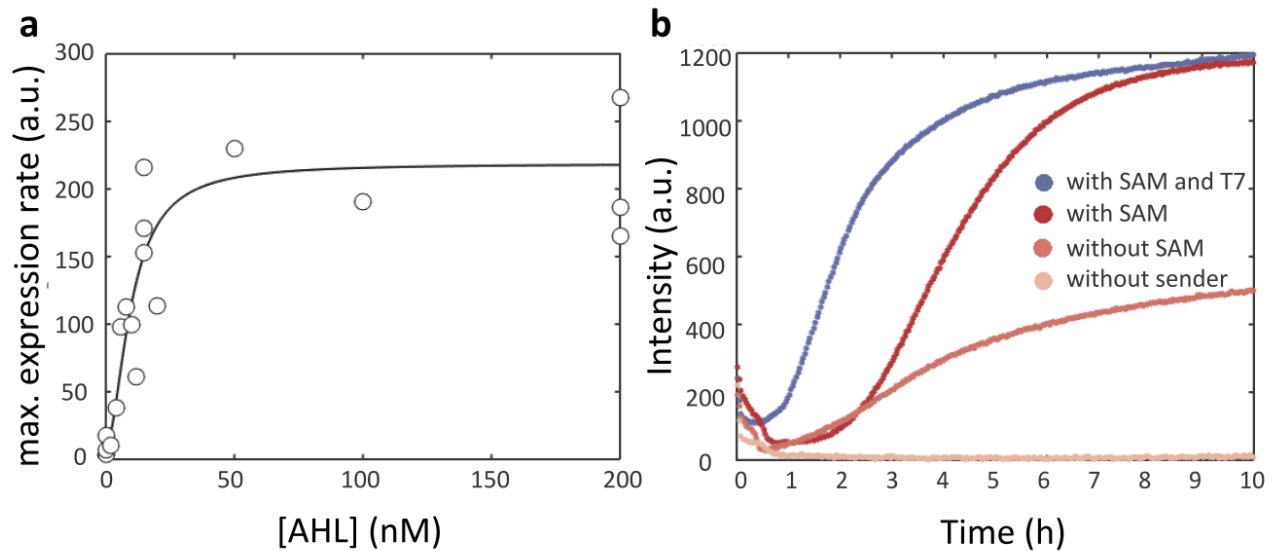


Figure S1: Bulk characterization experiments. **a)** Maximum GFP expression rate under control of the lux promoter for varying concentration of AHL. A fit with a Hill curve (continuous line) results in a Hill exponent of $n=1.8$ and an induction threshold at $K_a \approx 9.9$ nM. **b)** GFP production recorded in a bulk experiment with sender and receiver plasmids in a cell-free gene expression system. Addition of S-adenosyl methionine (a precursor for the production of the AHL 3-oxo-hexanoyl-L-homoserine lactone by LuxI) considerably increases the production of GFP. Addition of T7 RNA polymerase leads to faster production.

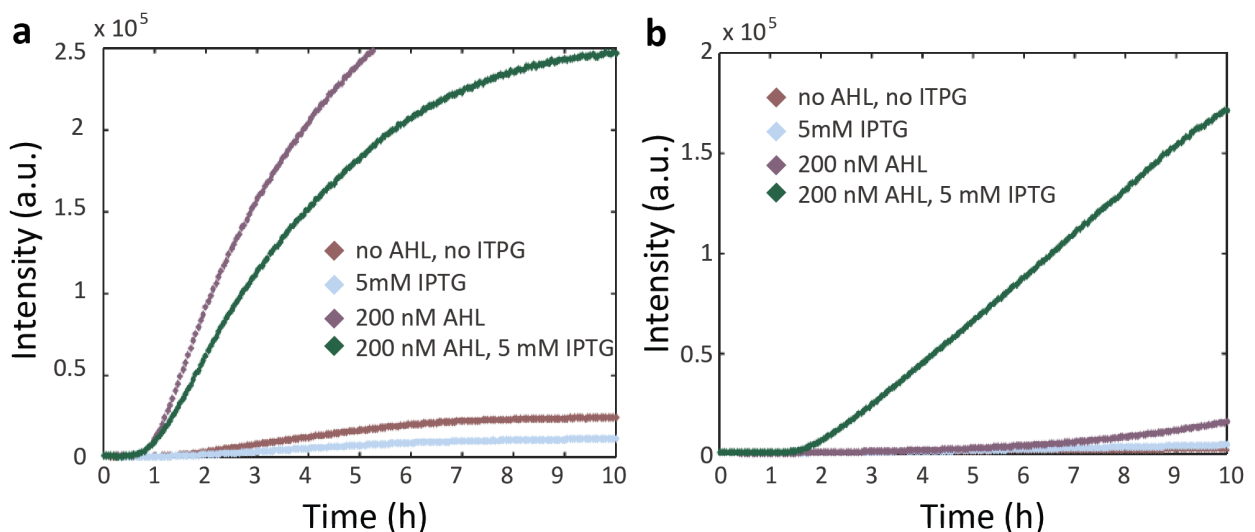


Figure S2: Bulk experiment with the AND gate in a cell-free system without and supplemented with LacI. **a)** GFP expression without addition of LacI. The cell free system contained AND gate plasmid at 7.5 nM concentration. The GFP production is clearly suppressed in the absence of AHL. However, IPTG only has a minor influence due to a shortage of LacI in the cell extract. **b)** Supplementing the cell free system with ≈ 200 nM His-tagged purified LacI suppresses the GFP production in the absence of either AHL or IPTG or both (bulk experiment with 7.5 nM of AND gate plasmid), and thus restores the AND gate behaviour with AHL and IPTG as input. This can also be achieved by reducing the plasmid concentration to about 1 nM (data not shown).

2. Diffusion of inducers in droplet emulsions

2.1 Analytical treatment

For an analytical description of our system, we first assume that the transport of the inducer AHL in our quasi-1D droplet emulsion can be treated as a simple diffusion process with an apparent diffusion coefficient D_a . The corresponding 1D diffusion equation for the concentration $a := [\text{AHL}]$ in the capillary reads:

$$\frac{\partial a}{\partial t} = D_a \frac{\partial^2 a}{\partial x^2}, \quad (1)$$

Assuming a constant AHL concentration a_0 at the end of the capillary in contact with the reservoir ($x = 0$) and zero concentration (infinitely) far inside the capillary, the boundary conditions are:

$$a(0, t) = a_0, \quad a(x \rightarrow \infty, t) = 0.$$

The initial concentration inside the capillary is zero: $a(x, 0) = 0, x > 0$.

The analytical solution to this semi-infinite system is the complementary error function:

$$a(x, t) = a_0 \cdot \text{erfc}\left(\frac{x}{\sqrt{4D_a t}}\right). \quad (2)$$

Thus, the distance $x(t)$ at which the AHL concentration has reached a given activation threshold K_a at time t is given by:

$$x(t) = \sqrt{4D_a t} \cdot \text{erfc}^{-1}\left(\frac{K_a}{a_0}\right). \quad (3)$$

If we assume that for very large reservoir concentrations a_0 the AHL concentration in any given droplet quickly sweeps from essentially zero to saturation (well above K_a), the time $t_{on}(x)$ at which GFP production starts is approximately given by the time at which the AHL diffusion front reaches the droplet (this will be true at least for the droplets closest to the reservoir). As shown in Figure S3b, we estimate $t_{on}(x)$ by measuring the time $t_1(x)$, it takes until certain GFP intensity is reached (arbitrarily set to 15% of the max. expression level). Additionally, we assume that it takes a time τ to reach this level at full induction. Then we have: $t_1(x) = t_{on}(x) + \tau$.

Accordingly, in Figure S3a, $x(t_1)$ was plotted against t_1 and then fitted with the power law:

$$x(t_1) = \alpha \cdot (t_1 - \tau)^\beta \quad (4)$$

(the exponent β accounts for potential anomalous processes). α is related to the apparent diffusion constant via

$$\alpha \approx \sqrt{4D_a} \cdot \text{erfc}^{-1}\left(\frac{K_a}{a_0}\right). \quad (5)$$

For $a_0 = 10 \mu\text{M}$, a fit to the data for the droplets close to the reservoir yields $D = 43 \frac{\mu\text{m}^2}{\text{s}}$ and $\beta = 0.49$, consistent with a normal diffusion process. When the same fit is performed only to data obtained from droplets at distances larger than (arbitrarily) $1500 \mu\text{m}$, the apparent diffusion coefficient is considerably reduced. As can be seen in Fig. S3b, the maximum GFP production rate slows down at large distances, and therefore GFP expression may not faithfully report the diffusion of AHL in the system. Potentially, at large distances the AHL concentration does not rise quickly enough to justify the assumption of an “all or none” expression. Furthermore, a reduced expression rate may be due to a degradation of the cell-free extract over time, which is felt more strongly at large distances.

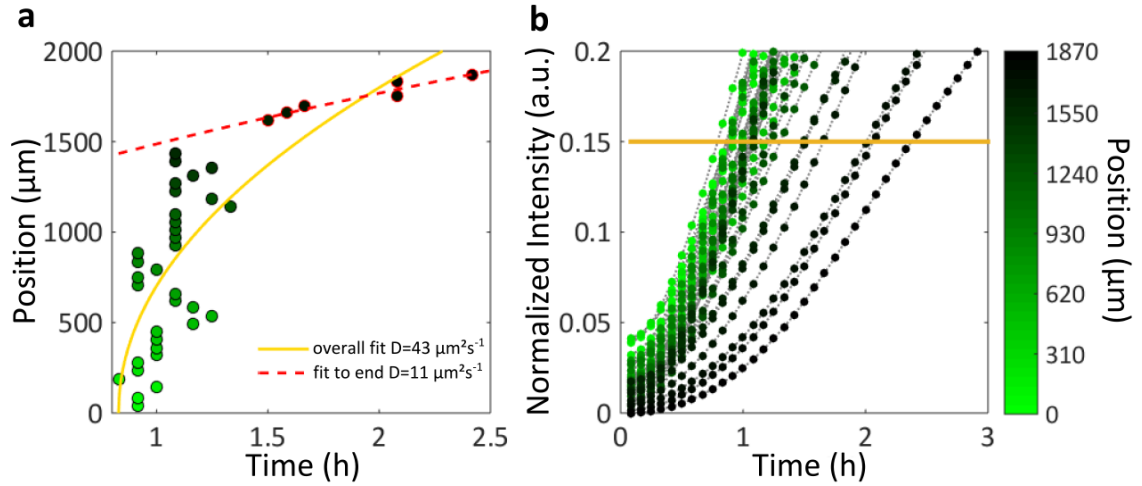


Figure S3 Estimation of D_a from data from the 10 μM AHL reservoir. a) A plot of position vs. the time t_1 at which 15% of total expression is reached (defined in (b)) is fitted with a power law (eq. (4)) to estimate the apparent diffusion constant. The orange line corresponds to a fit to all data points, whereas the dashed red line represents a fit (with fixed $\beta = 0.5$) to data points obtained at a distance larger than 1500 μm from the reservoir, indicated by red edges. **b)** t_1 is defined as the time when the time trace of a droplet at a certain position, indicated by the colour bar, has reached a level of 15% of the maximum intensity.

2.2 Reaction-diffusion modelling

In order to more accurately estimate the apparent diffusion coefficient than in the previous section, we have to account for the kinetics of GFP production in the droplets in greater detail. To this end, numerical simulations based on a simplified reaction-diffusion model and parameters determined from bulk experiments were performed and compared to the experimental data.

The model equations are:

$$\begin{aligned}\frac{\partial a}{\partial t} &= D_a \frac{\partial^2 a}{\partial x^2} - \delta_a a, \\ \frac{\partial g}{\partial t} &= \frac{\alpha_g(t) \cdot a^n}{K_a^n + a^n} - g \alpha_{mat}, \\ \frac{\partial g_{mat}}{\partial t} &= g \alpha_{mat}.\end{aligned}$$

Here, a , g and g_{mat} are the concentrations of AHL, nascent and matured GFP, respectively. D_a is the apparent diffusion coefficient of AHL and δ_a is its degradation rate. The Hill exponent n and the induction threshold K_a describe the transfer function and were determined in bulk experiments (Fig. S1). It was assumed that the GFP expression rate, $\alpha_g(t)$, in the cell extract exponentially approaches the expression rate of full induction, α_{max} (as shown by Karzbrun et al.¹), with $\alpha_g(t) = \alpha_{max}(1 - \exp[-(t - t_1)/t_2])$. The delay time in protein expression at the beginning is expressed by t_1 and the lifetime of mRNA molecules by t_2 . α_{max} effectively serves as a scaling variable and was adjusted in the simulations. Boundary and initial conditions were chosen as before.

The equations were solved numerically applying the MATLAB solver *pdepe*. Since the model does not account for depletion of resources in the cell extract it is only applied to the first $\approx 2\text{h}$ of the reactions. Additionally, a dead time of 5-40 min is assumed, corresponding to the dead time for the different experiments. Simulation parameters are listed in Table S2. As shown in Figure S4, the model can reproduce the observed kinetics quite satisfactorily. D_a and α_g as the only fit parameters essentially influence the ‘fanning out’ and scaling of the curves, respectively. We note that variation of the other parameters results primarily in changes of the reaction kinetics, i.e. the curvature of the time traces, but cannot account for their fanning out. The estimated apparent diffusion constant for $a_0 = 10 \mu\text{M}$ is within the range estimated before. Surprisingly, for lower reservoir concentrations (e.g., $a_0 = 1 \mu\text{M}$) the simulation matches

the experimental data only by setting an apparent diffusion coefficient that is decreased by about 2 orders of magnitude (Table S1).

Table S1: comparison of estimated apparent diffusion coefficients

a_0 (μM)	0.2	1	10	
method	RD	RD	analytical	RD
β	-	-	0.49	-
$D_{a,eff}$ ($\mu\text{m}^2\text{s}^{-1}$)	0.15	0.08	11-43	25

Table S2: parameters included in the simulations

a_0 (μM)	0.2	1	10	Source
$D_{a,eff}$ ($\mu\text{m}^2\text{s}^{-1}$)	0.15	0.08	25	adjusted
α_g (pM s^{-1})	400	180	280	adjusted
δ_a (s^{-1})	10^{-5}			(Hense, 2007) ²
α_{mat} (s^{-1})	$3.33 \cdot 10^{-3}$			(Iizuka, 2011) ³
n	1.8			bulk experiment
K_a (nM)	9.9			bulk experiment
t_1 (min)	20			(Karzbrun, 2011) ¹
t_2 (min)	12			(Karzbrun 2011) ¹

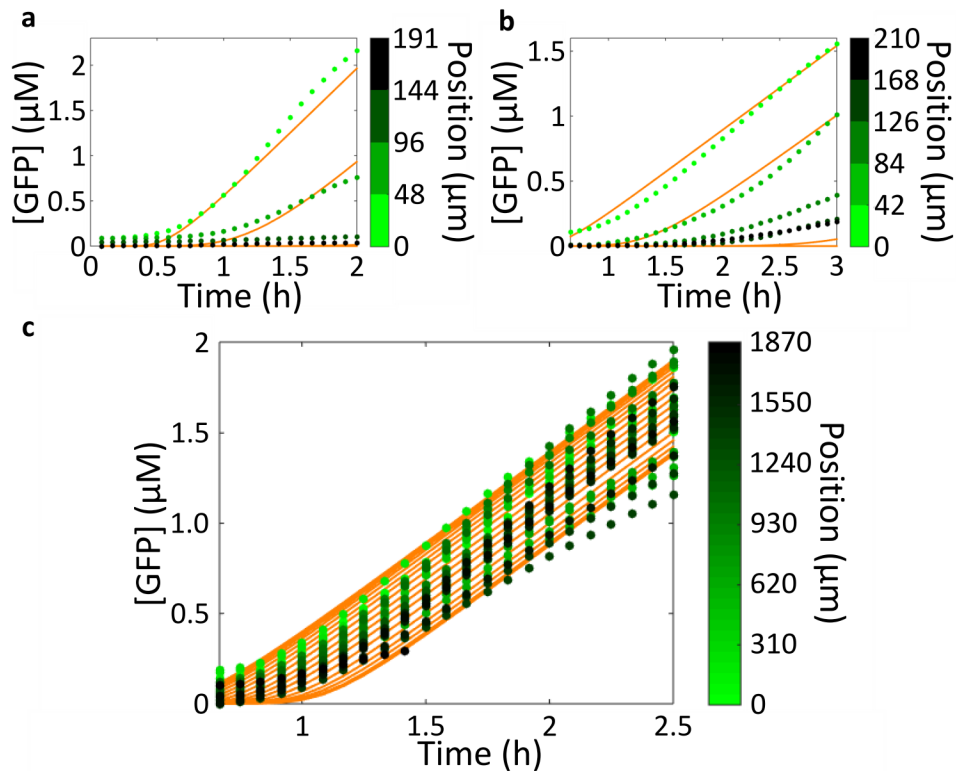


Figure S4: Estimation of apparent diffusion coefficients from the RD model. Orange lines represent simulations, while dots represent experimental data. Positions of droplets are indicated by colour bars. **a) $a_0=200$ nM, b) $a_0 = 1$ μM c) $a_0=10$ μM .** Simulation parameters are listed in Table S2. Experimental data can only be reproduced by using a ≈ 100 -fold higher apparent diffusion coefficient for $a_0=10$ μM compared to the lower reservoir concentrations. Note that the model was applied to simulate the initial phase of the reaction only, and the estimated dead time was 5 min for (a) and 40 for (b),(c), respectively.

2.3 Discussion

A variety of effects have to be considered that can potentially account for the reduced diffusion coefficients determined in the previous sections, in particular the discrepancy between the apparent diffusion coefficient for high and low reservoir concentrations:

- a) Depletion of resources and accumulation of waste products in the cell-free droplets
- b) Depletion of AHL by binding to LuxR, and degradation.
- c) Transport mechanisms in a water-in-oil emulsion.

a): Indeed we experimentally observe a change in the cell-free expression medium as indicated, e.g., by changes in pH (Figure S5), probably leading to a slowing down of GFP expression over time. Heuristically, this could be modelled by time dependent reaction constants in the model. In order to avoid complications caused by degradation, we applied the reaction-diffusion model only for the first ≈ 2 h of the reaction.

b): The apparent diffusion coefficient could indeed be concentration dependent if the diffusing molecules were immobilized along their path. Binding of AHL to LuxR occurs with a dissociation constant of ≈ 100 nM⁴ and an estimated unbinding rate of ≈ 10 min⁻¹⁵. In the cell-free system, LuxR is synthesized during the reaction, potentially reaching concentrations of a few μ M. Simulations taking reversible binding of AHL to LuxR into account could however not provide an explanation for the observed concentration dependent diffusion behavior.

c): In general, the apparent diffusion coefficient of small molecules in water-in-oil emulsions depends on the different diffusion coefficients in the oil and water phase, partitioning between the phases, interfacial properties and geometrical factors. In systems similar to ours (FC-40 oil and 'EA-surfactant') it was found that the permeability between droplets is primarily dependent on partitioning between the oil and water phase, which in turn depends on the surfactant concentration. The 'EA-surfactant' was designed for optimal emulsion stability and biocompatibility. It is not expected for this surfactant/oil system to form surfactant bilayers due to steric interactions between the relatively long triblock copolymers of perfluorinated polyethers (2000 – 6000 g/mol) which serve as tail groups⁶. Furthermore the tail groups are very well soluble in the continuous phase, reducing the adhesion energy between the monolayers⁷. Since the head groups are uncharged, it is unlikely that the stabilizing surfactant monolayer at the droplets boundary acts as a major barrier to diffusion^{8,9}.

In a simplified model, the apparent diffusion coefficient for a system composed of a droplet of radius r with diffusion coefficient $D_{aqueous}$ and an oil barrier of thickness h and diffusivity $D_{continuous}$ fulfills the relation:

$$\frac{L}{D_a} = \frac{2r}{D_{aqueous}} + \frac{h}{KD_{continuous}},$$

where $L = 2r + h$. The partition constant $K = \frac{c_{continuous,eq}}{c_{aqueous,eq}}$ describes the equilibrium concentrations at the oil-water interface. The permeability is defined as:

$$P = \frac{KD_{continuous}}{h}.$$

Typically the diffusion coefficient of small molecules such as AHL in aqueous solution is on the order of $D_w \approx 100 - 1000 \mu\text{m}^2/\text{s}$, which would be similar in M9 medium for droplets containing bacteria. Since the viscosity of the cell-free system should be comparable to that of the *E.coli* cytoplasm, the diffusion coefficient would be reduced by a factor between 2 and 5 in cell-free droplets. The same argument can be made for the diffusion coefficient in fluorinated oil, which has a 4-fold higher viscosity than water so that $D_{oil} \approx 25 - 250 \mu\text{m}^2/\text{s}$. Furthermore, a typical diffusion coefficient of surfactant micelles is about $D_{micelles} \approx 1 \mu\text{m}^2/\text{s}$ ⁸.

If we assume that $D_{aqueous} \gg LP$ and $L \approx 2r$, the apparent diffusion coefficient is dominated by the permeability, i.e., $D_a \approx LP = \frac{LK}{h} D_{continuous}$.

If the AHL concentration in the reservoir is very high, it is conceivable that the fraction of AHL partitioned into the oil phase (even for low K) is sufficient to induce gene expression. In this case, the apparent D_a "sensed" by the droplets will be proportional to $D_{oil} \approx 25 - 250 \mu\text{m}^2/\text{s}$. By contrast, for low inducer concentrations, AHL partitioning into oil

is too low, and AHL transport through micelles will dominate, i.e., $D_a \sim D_{micelles} \approx 1 \mu\text{m}^2/\text{s}$. This may in fact account for the observed factor of ≈ 100 between the apparent diffusion coefficients for low and high AHL.

3. pH changes in the cell-free transcription/translation system

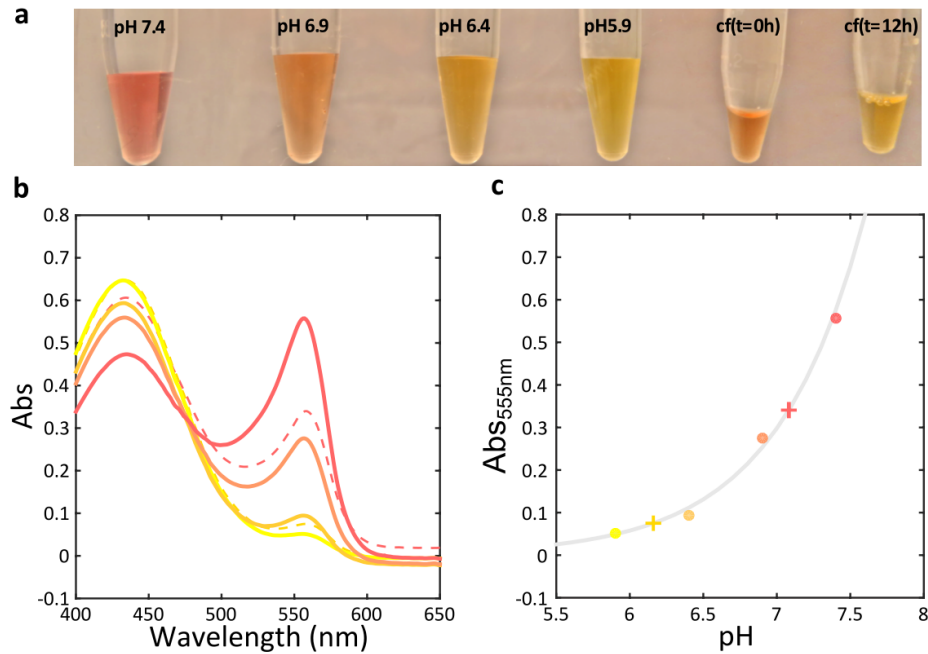


Figure S5: pH values in the cell free system tested with phenol red. a) Phenol red calibration solutions (100 μM in PBS) between pH 7.4 and pH 5.9 and cell-free reactions supplemented with 100 μM phenol red. **b)** Absorption spectra of calibration solutions and the cell free samples (dashed lines) at $t = 0\text{h}$ and $t = 12\text{h}$ after initiation of the experiment. **c)** Absorption maxima at 555 nm plotted against the corresponding pH values. Data points were fitted with an exponential curve.

4. Additional data – AHL and IPTG reservoir for bacteria and cell free system

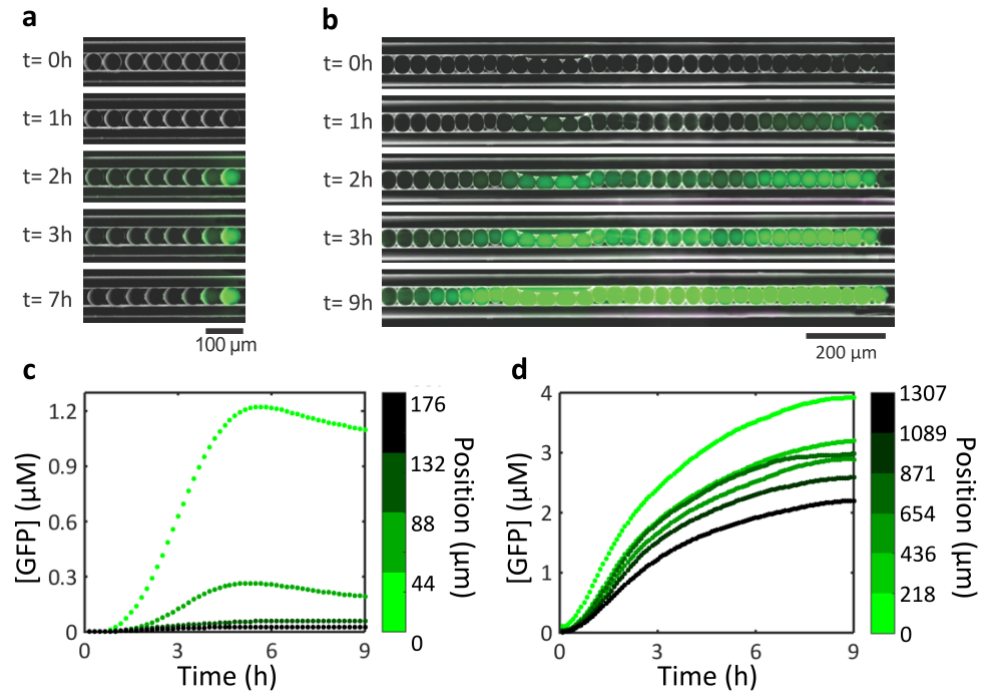


Figure S6: Cell-free droplets next to AHL reservoirs (alternative data set to main paper). **a)** Time series for a reservoir concentration of 200 nM. **b)** Time series for a reservoir concentration of $10\text{ }\mu\text{M}$. **c)** Average time traces from 3 capillaries for $a_0 = 200\text{ nM}$, positions indicated by the colour bar. **d)** Average time traces from 3 capillaries for $a_0 = 10\text{ }\mu\text{M}$. For clarity, time traces represent averages from groups of 5 neighbouring droplets.

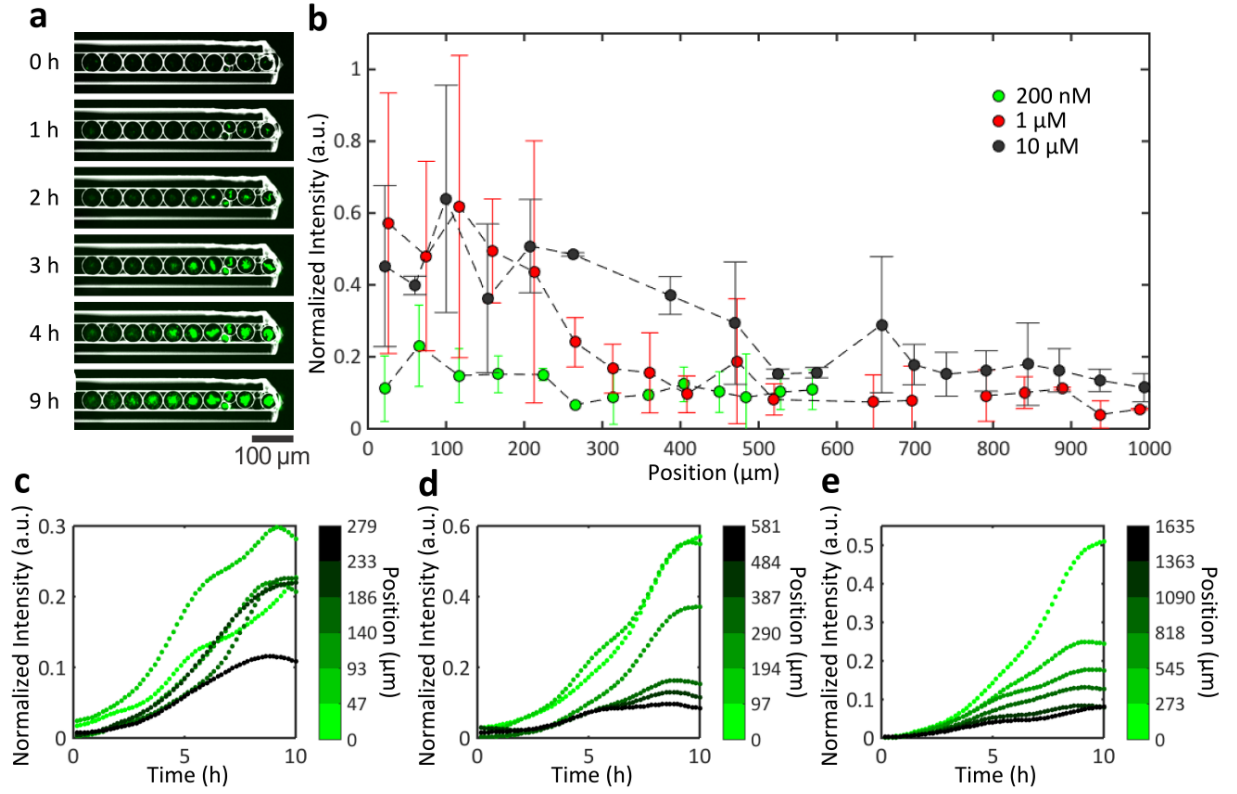


Figure S7: AND gate bacteria in droplets next to AHL reservoirs. **a)** Time series of bacteria containing droplets exposed to a reservoir concentration of 1 μM . Expression of GFP is sequentially induced as AHL diffuses into the capillary from right to left. **b)** Average fluorescence profiles for reservoir concentrations of 200 nM, 1 μM and 10 μM 9 h after initiation of the experiments. Error bars represent standard deviations from 3, 2 and 2 individual capillaries, respectively. **c)** Average time traces for the first 6 droplets next to a **200 nM** reservoir. Positions of droplets are indicated by colour bars., **d)** Average time traces for $a_0 = 1 \mu\text{M}$. For clarity, each trace represents the average of two neighbouring droplets. **e)** Average time traces for $a_0 = 10 \mu\text{M}$. Each trace represents the average of 6 neighbouring droplets.

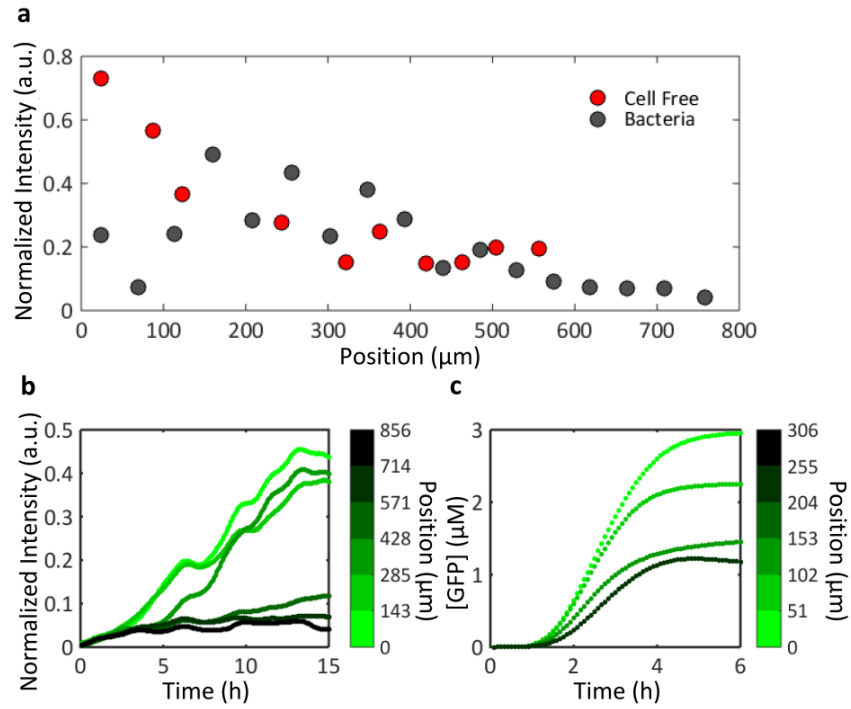


Figure S8: Cell-free and bacteria containing droplets next to 10mM IPTG reservoirs. a) Fluorescence profiles for cell-free and bacteria containing droplets 15 h after initiation of the experiments. **b)** Time traces for bacteria containing droplets, where positions are indicated by the colour bar. Data represents averages from 3 neighbouring droplets for a single capillary. **c)** Time traces for cell free droplets (data from a single capillary).

5. SI methods

5.1 Data processing

Microscope images were analysed with an automated droplet tracking software developed in the lab¹⁰. Time traces of cell-free droplets were generated by taking the mean fluorescence intensities over the whole droplet area (determined from bright field images). Since bacteria accumulate in the centre of the droplet, in this case the mean was taken only from bright pixels (defined by automated thresholding). Intensity traces were then normalized by subtracting the minimum of all traces of one capillary. Based on the distance of the droplets the time traces of multiple (2-3) capillaries were averaged. Subsequently, time traces were normalized to the maximum intensity for bacterial receivers. For cell-free receivers, a calibration with droplets containing purified GFP was performed. Finally, time traces were smoothed in MATLAB with the function *smoothn* ($S=100$) to reduce the noise and account for missing data points. For data spanning more than one observation area, i.e. 1 μM and 10 μM AHL reservoirs, images A and B were recorded with an overlap so that a reference droplet was present in each image. Accordingly, extracted data was stitched by transforming the positions of droplets in B to the coordinate system of A. It was ensured that droplets present in both images were not taken twice. Positions of droplets were calculated as the distance to the first droplet plus the radius of the first droplet. Pixel sizes of the cameras are 8 μm and 13 μm for IX71 and IX81, respectively. For data of hybrids, droplets were sorted manually dependent on their number of sender neighbours/their position inside the capillary and averaged.

5.2 Filling of the capillaries

Squared borosilicate glass capillaries with dimensions 50 μm x 50 μm x 5 cm (VitroCom, USA) were fixed by placing one end of the capillary between a freshly activated PDMS block and a glass slide (Fig. S9). The end of the capillary was positioned within a pre-punched hole in the PDMS block (diameter 1.25 mm), into which the droplet solution was pipetted. The capillaries were filled by manually applying pressure on the PDMS block. Filled capillaries were sealed with vacuum grease and nail polisher. For the experiments with several droplet species, we scanned for and then focused on appropriate droplet configurations.

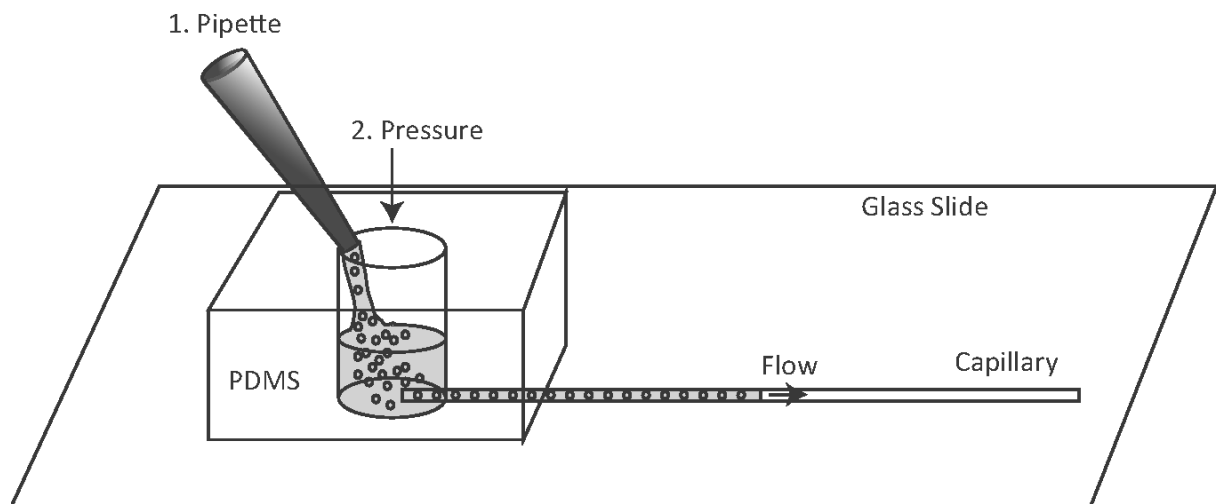


Figure S9: Filling of capillaries.

5.3 Plasmid maps

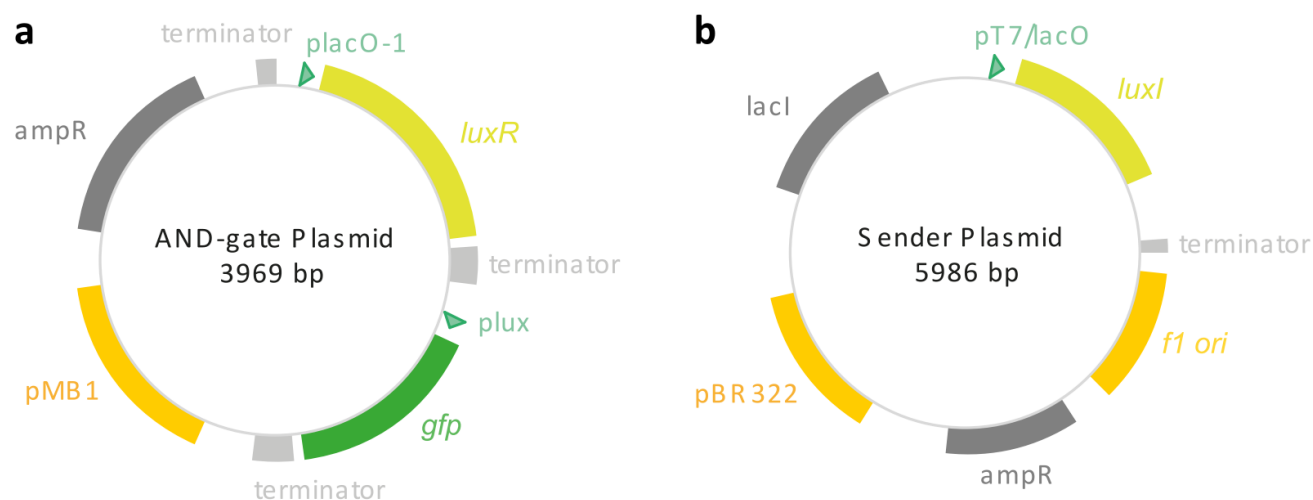


Figure S10: Plasmid Maps of constructs used in this study. a) The AND-gate plasmid is based on the BioBrick part pSB1A3 with insert T9002 completed with lacO-1 promoter. **b)** pETDuet-1 vector was used for the sender plasmid with the BioBrick insert C0061 (luxI). Construction details can be extracted from Weitz et al.¹¹

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