

# **Supplementary Material**

for

**Communication and quorum sensing in non-living mimics of eukaryotic cells**

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## Supplementary Methods

### *Fabrication of microfluidic chips*

A silicon wafer patterned with SU-8 photoresist served as a mold for PDMS devices. The mold was prepared following standard photolithography procedures to produce a feature height of 43  $\mu\text{m}$ . Design of the flow focusing junction was adapted from Desphande et al. 2016<sup>1</sup> (Fig. S1). PDMS (Sylgard, Dow Corning) was prepared at a 1:10 ratio and cured for 1h at 80°C. PDMS devices were bonded to PDMS spincoated cover glass using oxygen plasma (50 watt for 30 sec at 0.45 torr). After bonding, devices were baked overnight at 120°C to recover hydrophobicity. Channels downstream of the flow-focusing junction were rendered hydrophilic by a treatment with a 5% (wt/vol) polyvinyl alcohol (PVA) solution as described<sup>1</sup>. Following PVA-treatment, devices were baked again for 2 h at 120°C and used immediately or stored for up to two months.

### *DNA templates*

Plasmids used in this study are listed in Table S1. Plasmid DNA was purified using the NucleoBond Xtra Midi kit (Macherey-Nagel), followed by an isopropanol precipitation and resuspension of the DNA pellet in ultrapure H<sub>2</sub>O to prepare highly concentrated plasmid stocks and maximize expression in TX-TL reactions. The template for T3 RNA polymerase was on linear DNA prepared by PCR from a plasmid template. DNA template concentrations encapsulated in cell-mimics are listed in Table S2.

### *Cell-free transcription and translation reactions*

*E. coli* lysate for TX-TL reactions was prepared by freeze-thawing from *E. coli* BL21-Gold (DE3)/pAD-LyseR<sup>2</sup>. To induce T7 RNA polymerase activity besides *E. coli* RNA polymerase activity the main culture was induced with 0.5mM IPTG an optical density measured at 600 nm (OD<sub>600</sub>) of 0.5-0.6. Cells were harvested by centrifugation at an OD<sub>600</sub> of 1.4. Processing of the lysate was performed as described<sup>2</sup>. For the final composition of TX-TL reactions, cell lysate was diluted 2.5-fold with reaction buffer, microcapsules or DNA, and other additions as needed. This resulted in the following concentrations in the TX-TL reaction: 4.6 mg/ml protein, 7 mM Mg-glutamate, 60 mM K-glutamate, 3.5 mM DTT, 0.75 mM each amino acid except leucine, 0.63 mM leucine, 50 mM HEPES, 1.5 mM ATP and GTP, 0.9 mM CTP and UTP, 0.2 mg/mL tRNA, 0.26 mM CoA, 0.33 mM NAD, 0.75 mM cAMP, 0.068 mM folinic acid, 1 mM spermidine, 30 mM 3-PGA, 3.5% PEG-8000. When linear DNA templates were used, they were stabilized by adding 4  $\mu\text{M}$  of chi6 duplex DNA to the TX-TL reaction<sup>3</sup>. TX-TL reactions were incubated at 29°C for expression.

### *Preparation of labeled ribosomes*

*E. coli* ribosomes (New England Biolabs) were incubated in labeling buffer (50 mM HEPES pH 8.2, 100 mM KCl, 10 mM magnesium acetate) with a molar excess of Alexa Fluor 488 NHS Ester (ThermoFisher Scientific) for 90 min at room temperature. Free dye was removed by washing with labeling buffer in centrifugal filter devices with a molecular weight cut-off of 100 kDa. Each ribosome contained approximately eleven Alexa Fluor 488 labels.

### *Plate reader reactions*

Plate reader reactions were performed in 384-well plates using a 10  $\mu\text{l}$  reaction volume covered with 10  $\mu\text{l}$  light mineral oil in a Tecan infinite F200 plate reader. GFP fluorescence was read

every 5 min using a  $485 \text{ nm} \pm 20 \text{ nm}$  excitation filter and a  $550 \text{ nm} \pm 35 \text{ nm}$  emission filter, followed by 1 min of shaking. Fluorescence intensity measurements were calibrated using purified sfGFP-His<sub>6</sub> to determine absolute concentrations.

#### *Image analysis*

Fluorescence traces or endpoint intensities of individual hydrogel nuclei were extracted from timelapse movies by measuring fluorescence in manually selected oval regions in nuclei, using a non-fluorescent region in each cell-mimic for background subtraction in Fiji/ImageJ<sup>4</sup>. Artificial quorum sensing data was analyzed using the colony counter plugin in Fiji/ImageJ to segment and count cell-mimics and polymer beads in the stitched brightfield image of a droplet. When necessary, regions of interest were manually added or deleted. Fluorescence values of individual cell-mimics were mean fluorescence values of the individual segmented cell-mimics. For background correction, fluorescence values of segmented regions from each droplet were sorted. The lowest fluorescence intensities were from solid polymer beads, and we used this property for background correction. We removed the lowest 34 % of values, which was the percentage of polymer beads in the sample, and used the highest of the removed values for background subtraction of the reduced list. Experiments (each analyzed droplet with different amounts of cell-mimics) were performed for densities between 25 and 800 cell-mimics per droplet, which were binned every 50 cell-mimics. Each bin contained data from at least 156 analyzed cell-mimics, from one to seven experiments.

#### *Scanning electron microscopy*

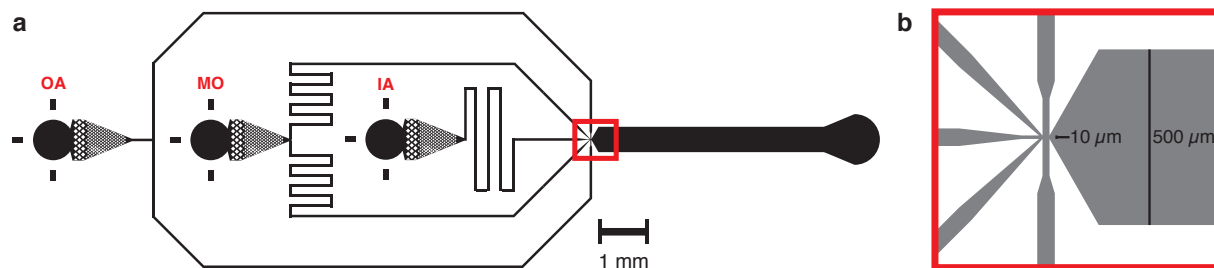
Scanning electron microscopy was performed with a Zeiss SIGMA VP field emission scanning electron microscope using air-dried cell-mimics. To image cross sections of microcapsule polymer membranes, cell-mimics were cut using a razor blade.

**Table S1. DNA templates used in this study**

<b>Name</b>	<b>Function</b>	<b>Description</b>	<b>Reference</b>
240x <i>tetO</i> array	Array of 240 TetR binding sites ( <i>tetO</i> ), PRS316-240xtetO (Addgene #44755)	Plasmid, high copy number, Ampicillin	Finney-Manchester et al. (2013) <sup>5</sup>
pT7- <i>tetR</i> -sfGFP	T7 promoter driven synthesis of C-terminal fusion of sfGFP to TetR repressor (TetR-sfGFP)	Plasmid, pTNT vector (Promega), high copy number, Ampicillin	This work
pT7- <i>tetR</i> -mCherry	T7 promoter driven synthesis of TetR-mCherry fusion protein	Plasmid, pTNT vector (Promega), high copy number, Ampicillin	This work
pT3- <i>tetR</i> -sfGFP	T7 promoter driven synthesis of TetR-sfGFP	Plasmid, pSB1C3 (Registry of Standard Biological Parts), high copy number, Chloramphenicol	This work
pT7-T3RNAP	T7 promoter driven synthesis of T3 RNA polymerase (only used as PCR template)	Plasmid, Low copy number, pSC101 origin, Kanamycin	This work
pT7-T3RNAP	T7 promoter driven synthesis of T3 RNA polymerase	Linear DNA, PCR amplification of functional region from pT7-T3RNAP plasmid	This work

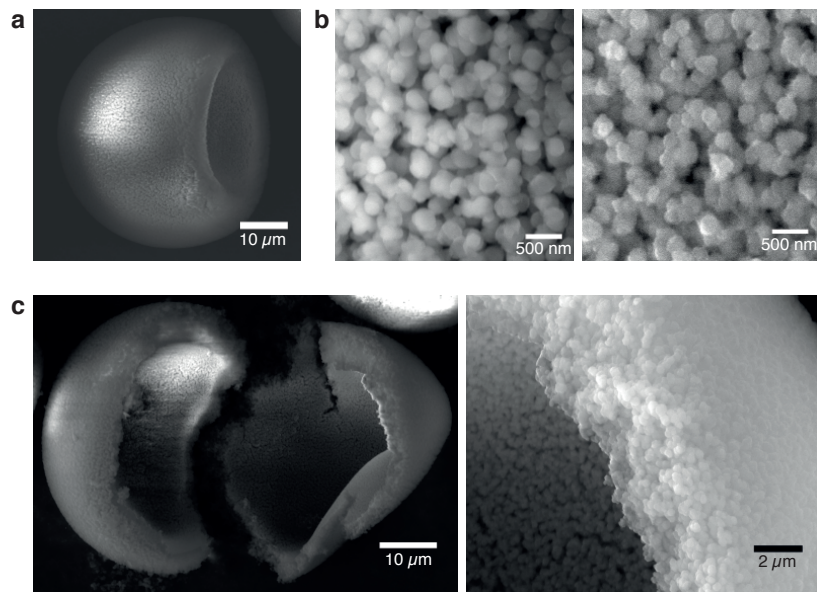
**Table S2. DNA content of cell-mimics used in this study.** Listed are DNA concentrations used in inner aqueous phase for double emulsion production.

<b>Name and Description</b>	<b>DNA concentrations</b>
<i>tetR</i> -sfGFP / <i>tetO</i> Production and capture of TetR-sfGFP	50 nM pT7_ <i>tetR</i> -sfGFP plasmid 20 nM 240x <i>tetO</i> array plasmid
<i>tetR</i> -mCherry / <i>tetO</i> Production and capture of TetR-mCherry	50 nM pT7_ <i>tetR</i> -mCherry plasmid 20 nM 240x <i>tetO</i> array plasmid
Sender cell-mimics Production of TetR-sfGFP	100 nM pT7_ <i>tetR</i> -sfGFP plasmid
Receiver cell-mimics Capture of TetR-sfGFP	20 nM 240x <i>tetO</i> array plasmid
Activator cell-mimics Production of T3 RNAP	40 nM pT7_T3RNAP linear DNA
Reporter cell-mimics T3 promoter controlled production and capture of TetR-sfGFP	30 nM pT3_ <i>tetR</i> -sfGFP plasmid 20 nM 240x <i>tetO</i> array plasmid
1-color density sensors cell-mimics Artificial quorum sensing	2.5 nM pT7_T3RNAP linear DNA 30 nM pT3_ <i>tetR</i> -sfGFP plasmid 15 nM 240x <i>tetO</i> array plasmid
2-color density sensor cell-mimics Artificial quorum sensing with 2-color response	2.5 nM pT7_T3RNAP linear DNA 20 nM pT3_ <i>tetR</i> -sfGFP plasmid 20 nM pT7_ <i>tetR</i> -mCherry plasmid 15 nM 240x <i>tetO</i> array plasmid



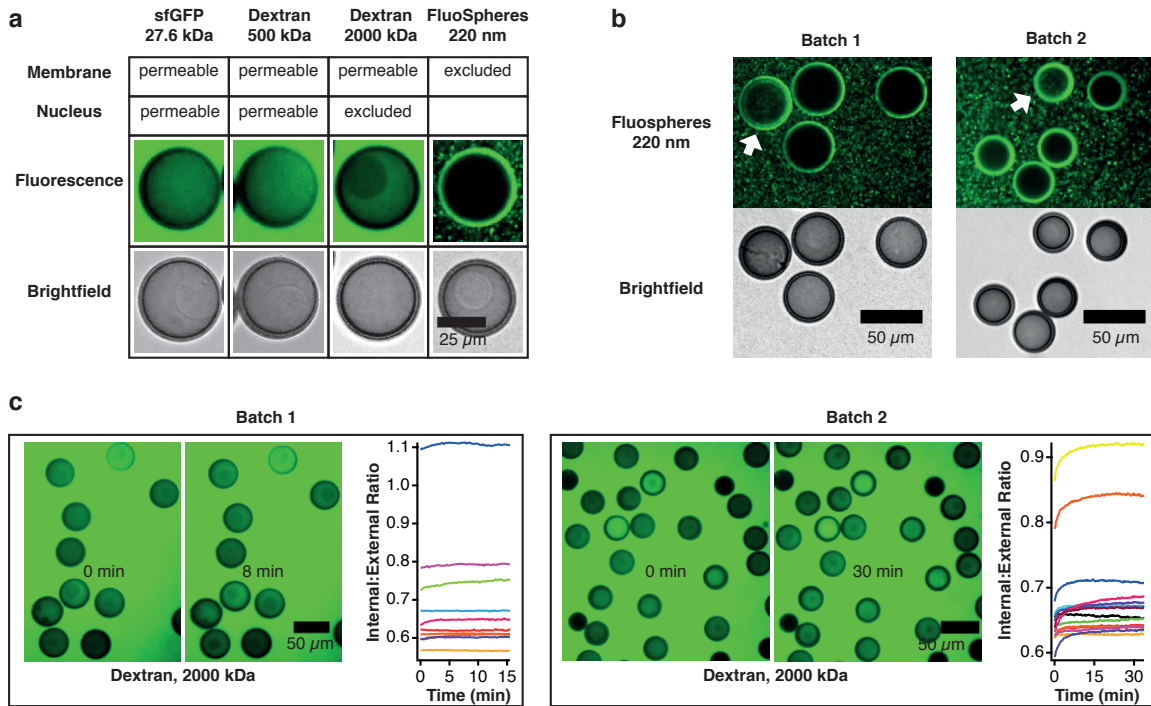
**Supplementary Figure S1. Design of the microfluidic device.**

a) Design of the microfluidic device used to produce cell-mimics. Inlets for the outer aqueous (OA), middle organic (MO) and inner aqueous phases (IA) are labeled. A magnified view of the flow focusing junction (red box) is shown in b). At the fluidic junction the width of the IA channel is 10  $\mu\text{m}$ .



**Supplementary Figure S2. Scanning electron microscopy of porous polymer membrane of cell-mimics.**

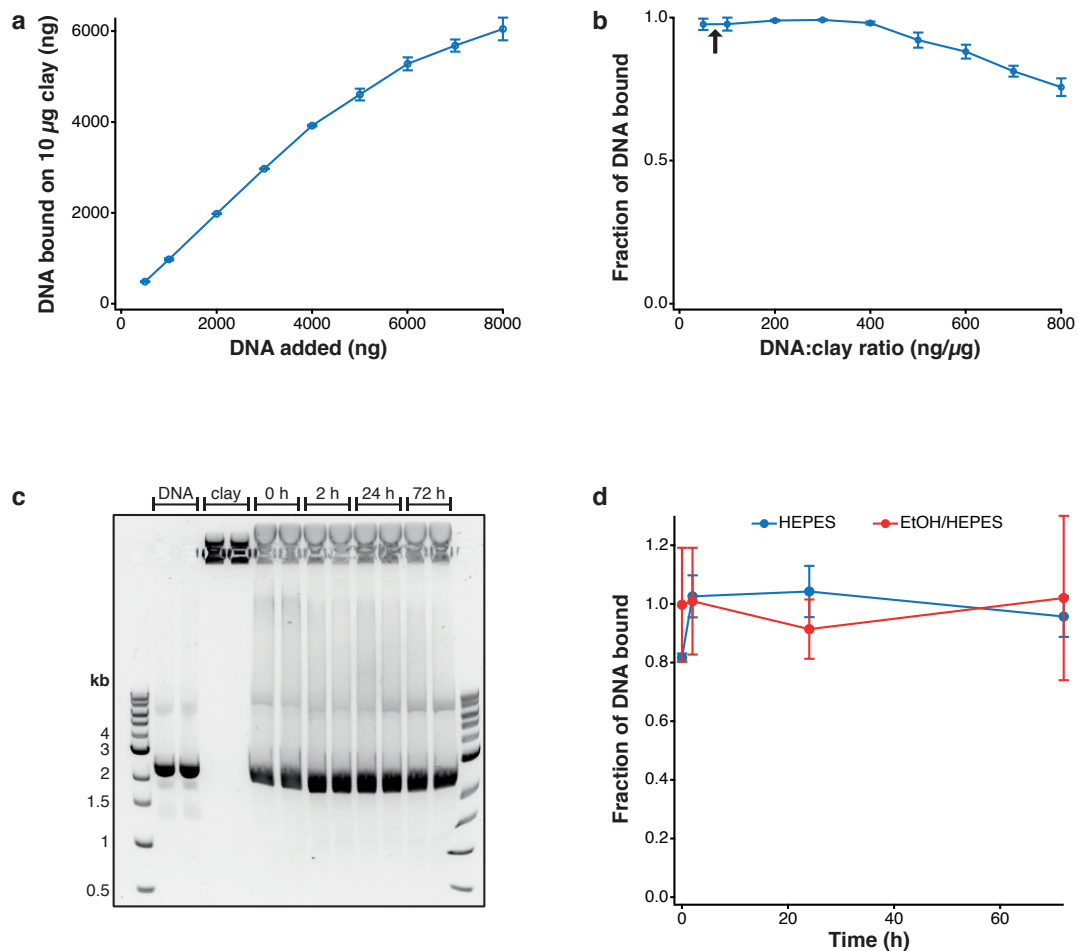
a) Low magnification image of cell-mimic with an indentation. Polymer membranes partly collapsed when cell-mimics dried, which caused indentations in the membrane. b) High magnification images of exterior of microcapsule membranes from two separate batches of cell-mimics. c) Low magnification image of a cracked cell-mimic and magnification of the polymer membrane cross-section.



### Supplementary Figure S3. Permeability of cell-mimic membranes and clay-DNA hydrogel nuclei.

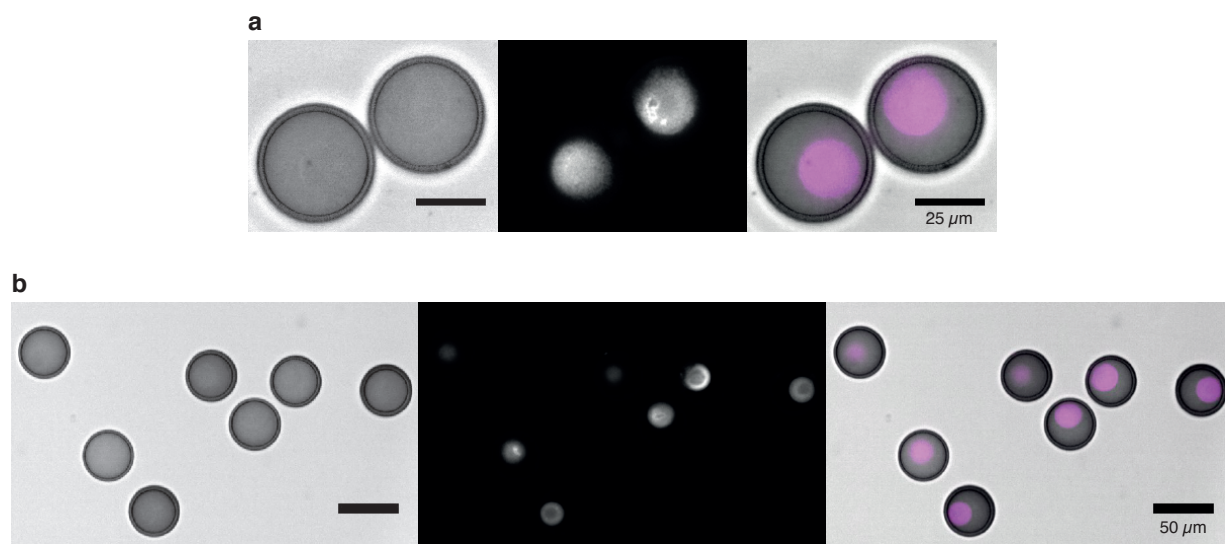
a) Spinning disk confocal fluorescence images of individual cell-mimics incubated for 24 h with fluorescent tracer molecules of different sizes (top) and corresponding brightfield images (bottom). b) Cell-mimics after incubation with a solution of 220 nm FluoSpheres (Thermo Fisher Scientific). FluoSpheres nanoparticles were excluded from 90% of cell-mimics ( $n=77$ , batch 1;  $n=152$ , batch 2). Arrows indicate cell-mimics with FluoSpheres in their interior. Many of the cell-mimics with nanoparticles in their interior had visible defects (see brightfield image, batch 1). Images of cell-mimics in a) and b) were acquired after 24 h incubation with labeled tracer molecules in 100 mM HEPES, 0.4% Tween 20. Dextrans were Fluorescein conjugates (Nanocs). c) Diffusion of 2000 kDa dextran into cell-mimics. At time 0, labeled 2000 kDa dextran was added and mixed with cell-mimics in 100 mM HEPES, 0.4% Tween 20. Images were acquired every 15 seconds to monitor diffusion of dextran into the interior of cell-mimics. Fluorescence images show a population of cell- at time 0 and after equilibration of fluorescence signals for cell-mimic batches 1 and 2. Graphs show traces of internal to external fluorescence ratios for individual cell-mimics tracked over time in the shown images. Diffusion of dextran into cell-mimics in batch 2 was slightly slower than for batch 1. An increase in fluorescence could be observed in most batch 2 cell-mimics, while in batch 1, fluorescence levels had already equilibrated at the start of imaging. Final fluorescence intensities varied from cell-mimic to cell-mimic probably because their polymer shells absorbed light to different extends. We observed similar differences in final intensities between cell-mimics for smaller the fluorescent tracer molecules sfGFP and 500 kDa dextran as well. The saturating fluorescence traces show that internal and external 2000 kDa dextran concentrations were equilibrated after 15 min at the latest.





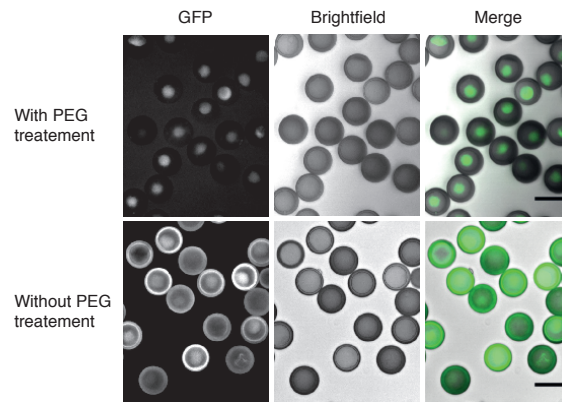
**Supplementary Figure S4. Characterization of DNA binding by Laponite XLG clay outside of cell-mimics.**

a) Binding curve of plasmid DNA on 10 µg clay. b) Fraction of DNA bound calculated from the same experiment. In cell-mimics, we used DNA at a maximum of 75 ng per µg clay as indicated by the arrow. Binding was measured by titrating plasmid DNA added to 10 µg of Laponite XLG in solution. Hydrogel formation was induced by addition of 200 mM KCl to a final volume of 10 µl. Hydrogel was removed by centrifugation and the DNA left in solution was measured photometrically. Binding experiments were performed in triplicates. Error bars show the standard deviation of the experimental repeats. c, d) Agarose gel analysis of DNA capture and retention under nucleus-formation and cell-mimic storage conditions. Clay-DNA hydrogel was formed at a ratio of 75 ng plasmid DNA per µg clay in 70% ethanol and 200 mM HEPES pH 8. Clay-DNA pellets were stored either in 150 µl 100 mM HEPES pH 8 or in 150 µl 70% ethanol, 200 mM HEPES pH 8 at 4°C for different times to analyze retention of DNA in the hydrogel. Before analysis on an agarose gel, supernatants were removed and hydrogel pellets homogenized. Experiments were performed in duplicate, and each sample was analyzed on two separate gels. c) Example gel for storage in HEPES buffer. The first four lanes were loaded with DNA or clay only. Each lane contained 4 µg clay and 300 ng plasmid DNA. d) DNA amounts in the samples were determined from band intensities on the gel. Error bars are standard deviations of experimental repeats.



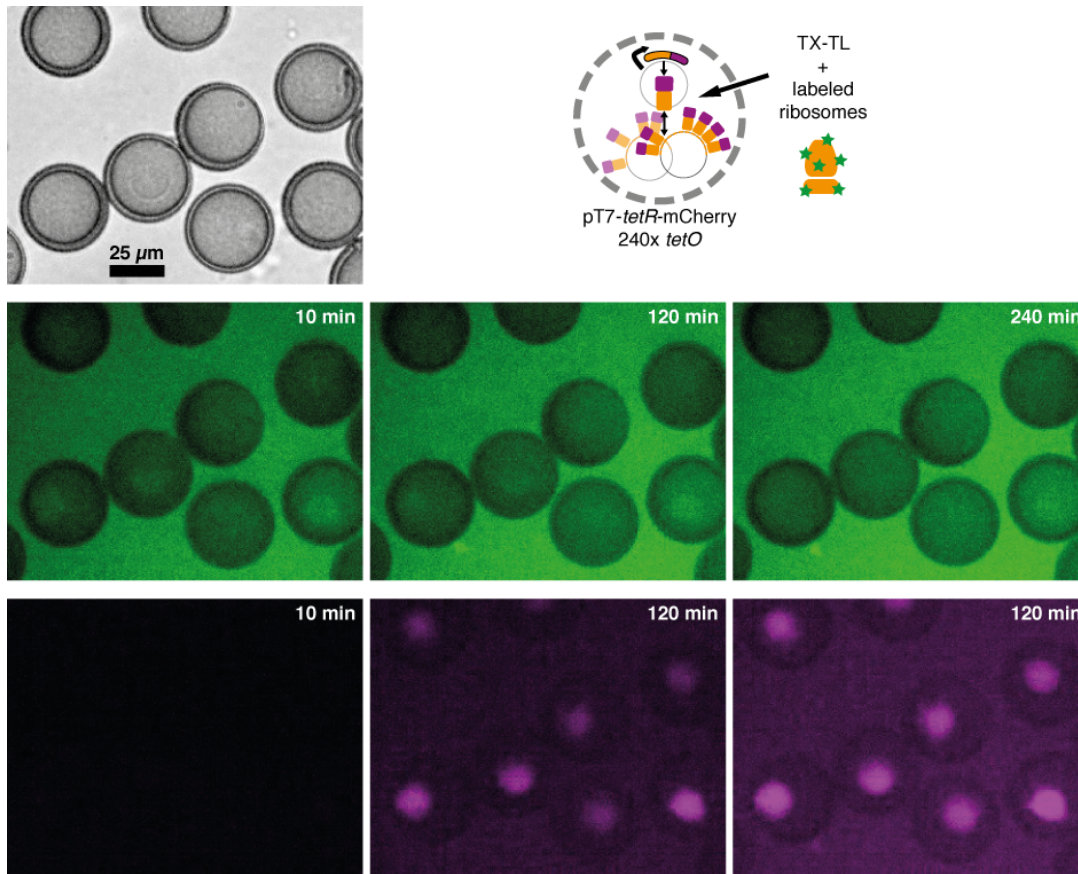
**Supplementary Figure S5. Morphologies of clay-DNA nuclei.**

a) Examples of clay-DNA hydrogel nuclei morphologies. Shown is a brightfield image (left), GelRed fluorescence of the clay-DNA nucleus (center) and a merged image (left). b) Variability of GelRed fluorescence in hydrogel nuclei. Intensity of dim hydrogel nuclei did not increase by focusing on a different section in cell-mimics.



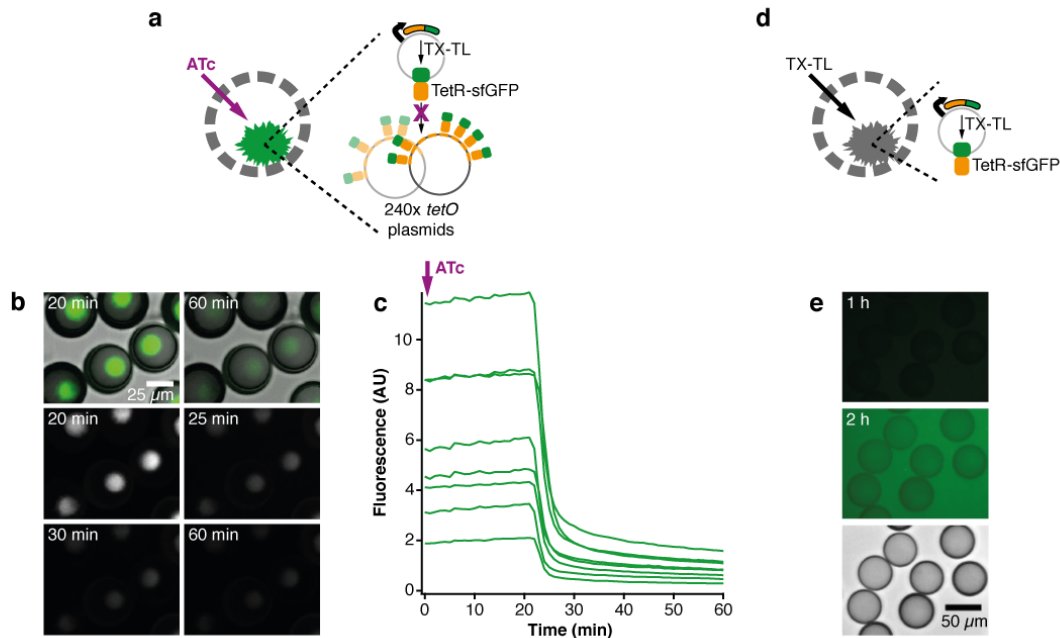
**Supplementary Figure S6. PEG treatment prevents non-specific binding of proteins to polymer membranes of cell-mimics.**

Endpoint fluorescence of *tetR*-sfGFP / *tetO* cell-mimics (Fig. 1c) and localization of TetR-sfGFP after 3 h TX-TL with or without PEG treatment.



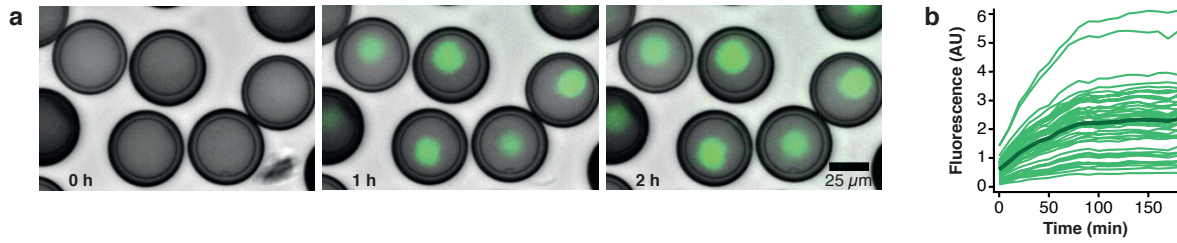
**Supplementary Figure S7. Cell-mimics' porous polymer membranes are permeable to ribosomes.**

Protein expression in *tetR*-mCherry / *tetO* cell-mimics was initiated by addition of TX-TL reagents spiked with Alexa Fluor 488 labeled *E. coli* ribosomes. Top shows a brightfield image and a schematic of the experiment. Alexa Fluor 488 labeled ribosomes (middle, green) could be detected in cell-mimics interior and in many cell-mimics their concentration was slightly increased in hydrogel nuclei over the rest of the interior. Hydrogel nuclei accumulated TetR-mCherry fluorescence over time (bottom, magenta).



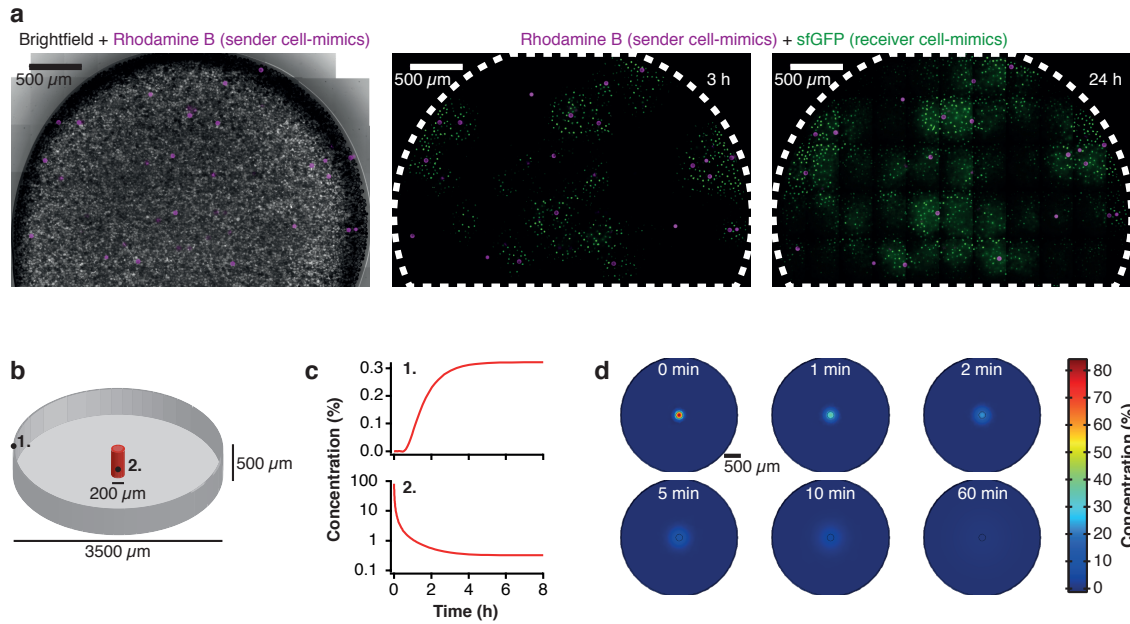
### Supplementary Figure S8. Specificity of TetR-sfGFP binding to *tetO* sites in hydrogel nuclei.

a-c) Anhydrotetracycline (ATc) induced unbinding from hydrogel nuclei containing 240x *tetO* plasmid. a) Schematic of the experiment. TetR-sfGFP was expressed in cell-mimics in TX-TL as in Fig. 1d. When expression ended after 3 h, cell-mimics were transferred into 200 mM HEPES pH 8, 2 mg/ml BSA. To observe ATc induced unbinding from nuclei, cells in 18  $\mu$ l of buffer were pipetted into a cylindrical reaction chamber prepared by punching a 2 mm hole into a 10 mm high block of PDMS, which was placed on cover glass for imaging. ATc for a final concentration of 2.5  $\mu$ M was pipetted on top of the liquid without active mixing. Imaging was started immediately and ATc was left to diffuse to cell-mimics. b) Timelapse images of TetR-sfGFP unbinding. Shown are merged images before and after unbinding (top) and GFP fluorescence images of the fast disappearance of TetR-sfGFP from hydrogel nuclei. c) Dynamics of the unbinding process observed by tracking fluorescence in eight representative hydrogel nuclei over time. d) Schematic of control experiment of *tetR*-sfGFP expression in cells without 240x *tetO* array plasmid. e) Timelapse images of GFP fluorescence (top, middle). To show increase of fluorescence in solution, images were set to a higher brightness than images in panel b. Bottom: brightfield channel.



**Supplementary Figure S9. Low batch-to-batch variability of protein expression in cell-mimics.**

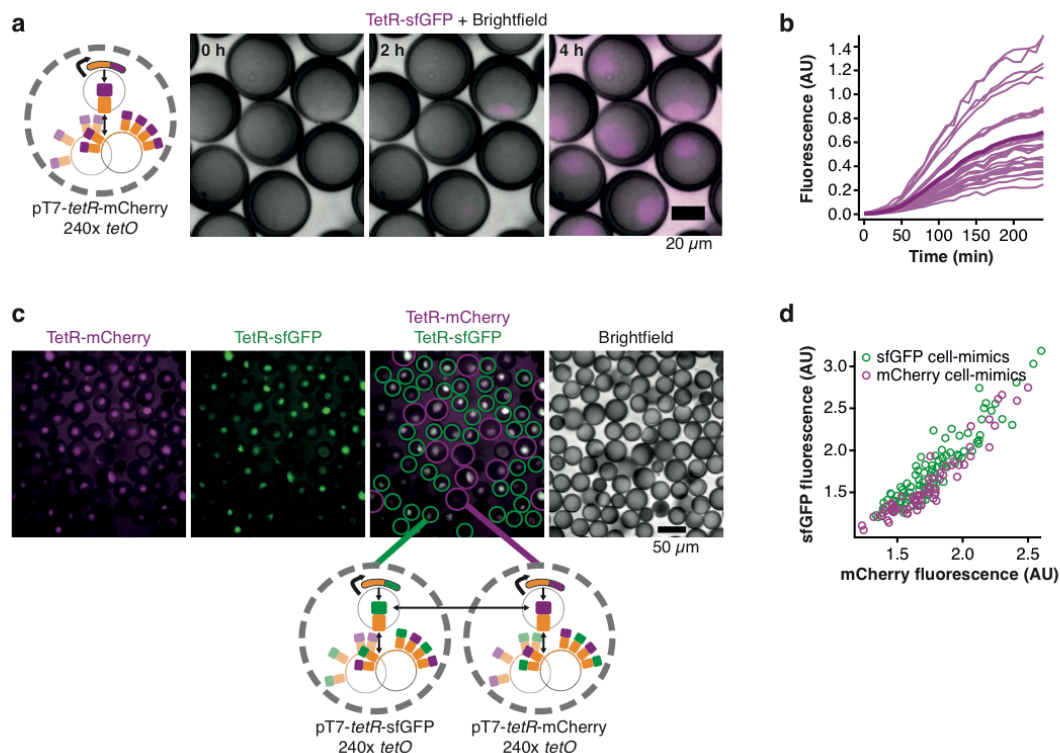
Expression and capture of TetR-sfGFP in a batch of cell-mimics prepared approximately a year later than those shown in Fig. 1. Timelapse images of TetR-sfGFP fluorescence increase in nuclei (green fluorescence merged with brightfield) and b) traces of fluorescence increase in 40 nuclei and averaged fluorescence (bold line).



**Supplementary Figure S10. Protein exchange in a large droplet of sender and receiver cell-mimics.**

a) Brightfield image of dense sender and receiver cell-mimic colony shown in Fig. 2b, merged with fluorescence from sender cell-mimics (magenta). Distribution of TetR-sfGFP fluorescence (green) is shown after 3 h and 24 h with identical brightness settings. b-d) COMSOL simulation of free diffusion of TetR-sfGFP (50.1kDa) in a geometry comparable to the experiment in a). We assumed a diffusion coefficient of  $6 \cdot 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ , which was measured for 67 kDa bovine serum albumin<sup>6</sup>. b) Schematic and dimensions of the geometry used in the simulation. Protein was initially located in a cylindrical region (red) in the center. Protein could freely diffuse in the entire grey cylinder, which was closed on the top and bottom. c) Concentration change in the two positions indicated in b). d) Surface concentration across the geometry at different time points.

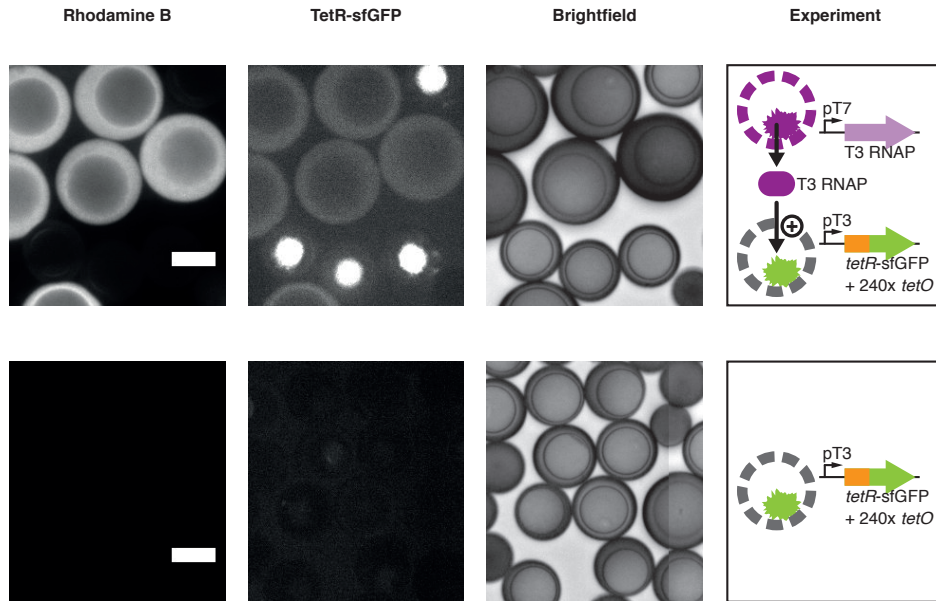




**Supplementary Figure S11. Protein exchange between TetR-mCherry and TetR-sfGFP producers.**

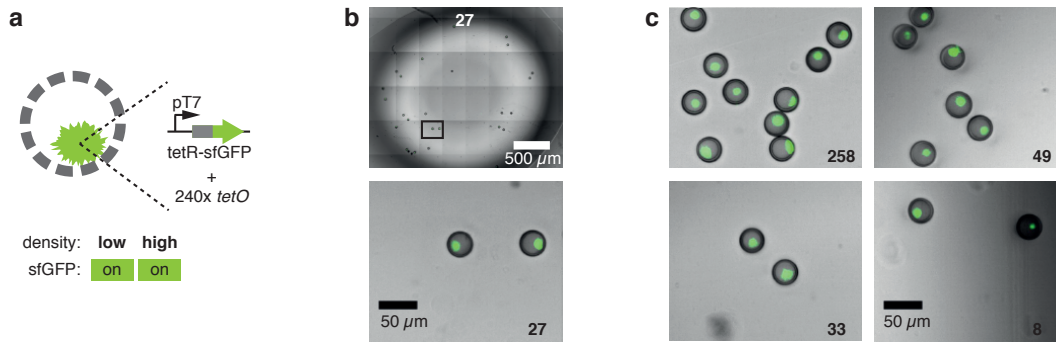
a) Schematic of cell-mimics expressing and binding TetR-mCherry (*tetR-mCherry* / *tetO*) and time lapse images of TetR-mCherry fluorescence (magenta) merged with brightfield images. b) Traces of fluorescence increase in hydrogel nuclei of 27 *tetR-mCherry* / *tetO* cell-mimics with the average in bold. c) Endpoint fluorescence (4 h) in a mix of *tetR-sfGFP* / *tetO* and *tetR-mCherry* / *tetO* cell-mimics. As shown in the schematic below, both cell-mimics types contain the 240x *tetO* plasmid and bind a mix of both fluorescent reporter proteins. Images show fluorescence channels separately and a merge of both fluorescence channels with circles indicating the positions of TetR-sfGFP producers (green) and TetR-mCherry producers (magenta), which were identified from the brightfield image by their difference in size and cell wall thickness. d) Correlation of sfGFP and mCherry fluorescence in TetR-sfGFP producers (green) and TetR-mCherry producers (magenta). Each data point shows sfGFP and mCherry fluorescence in the hydrogel nucleus of an individual cell-mimic. The cell-mimic types cannot be distinguished from each other by their respective fluorescence levels. Cell-mimics that could not be classified into a category based on their appearance in the brightfield channel were not analyzed.





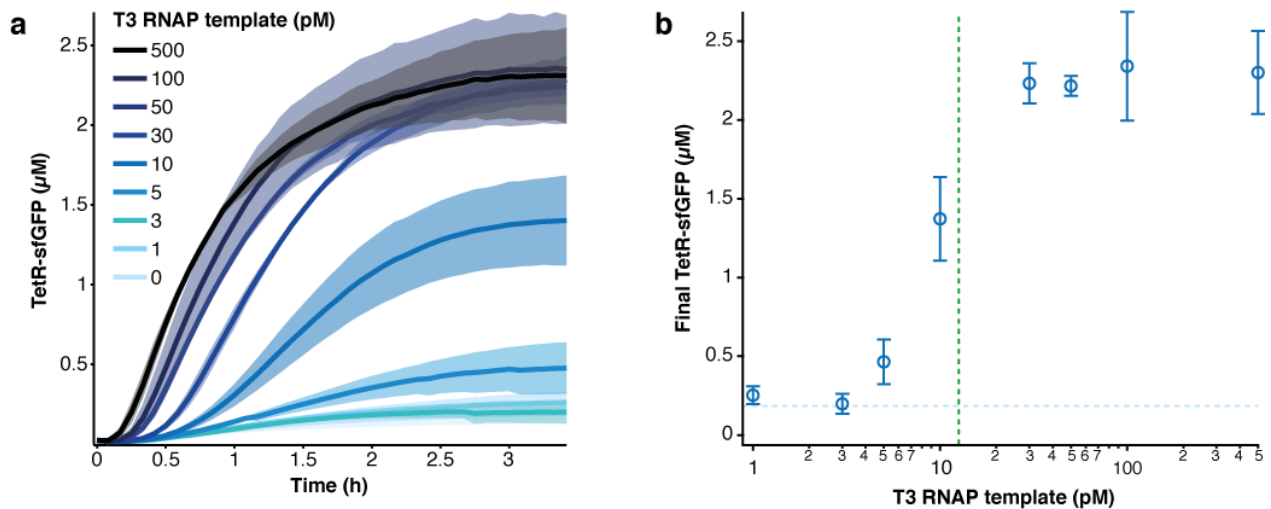
**Supplementary Figure S12. Activation of reporter cell-mimics by T3 RNAP producing activator cell-mimics and control experiment.**

Images of endpoint fluorescence (3 h) and brightfield channel from activation experiment with a mix of activator and reporter cell-mimics as well as a control experiment using reporter cell-mimics only as shown in the schematic. Activator cell-mimics were labeled with Rhodamine B in their polymer membranes.



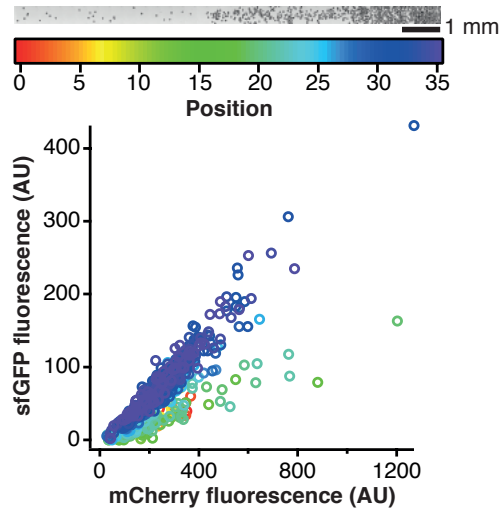
**Supplementary Figure S13. Constitutive producers of TetR-sfGFP show the reporter protein in their hydrogel nuclei at low cell-mimic densities.**

a) Schematic of control cell-mimics for the artificial quorum sensing experiments (Fig. 4) that express the reporter protein under control of a constitutive T7 promoter and contain the 240x *tetO* plasmid. Independent of cell density, control cells accumulated reporter protein in their nuclei. Constitutively expressing *tetR*-sfGFP / *tetO* control cell-mimics cell-mimics were as in Fig. 1C-D. b) Experiments were performed like in Fig. 4 in droplets of 4.5  $\mu$ l TX-TL containing different numbers of control cell-mimics as indicated. Lower image is a magnification of the region highlighted above. c) Magnifications of representative control cells in droplets containing the indicated number of cells. All images were set to identical brightness settings.



**Supplementary Figure S14. Titration of T3 RNAP template DNA in batch reactions.**

a) Dynamics of TetR-sfGFP production from a T3 promoter in TX-TL reactions containing different amounts of T3 RNAP template DNA. GFP fluorescence was read on a platereader in reactions containing 5 nM pT3-tetR-sfGFP reporter plasmid and variable amounts of pT7-T3RNAP linear template DNA. All reactions were done at least in triplicate and shaded areas indicate standard deviations between experimental repeats. b) Final TetR-sfGFP produced in a) as a function of T3 RNAP template concentration. Light blue dashed line indicates fluorescence of a reaction with no T3 RNAP template. Green dashed line indicates calculated T3 RNAP template concentration in a 4.5  $\mu\text{l}$  droplet containing 400 artificial quorum sensing cell-mimics, which was the lowest density at which expression of the reporter was observed in Fig. 4c.



**Supplementary Figure S15. Crowded and dilute 2-color density sensors display sfGFP to mCherry fluorescence in two distinct populations.**

Fluorescence of individual cell-mimics in the density gradient experiment shown in Fig. 4e is plotted as sfGFP versus mCherry fluorescence. Positions of individual cell-mimics in the gradient are color coded as shown in the color legend and the corresponding image above.

### **Movie 1. Production of double emulsion droplets.**

Timelapse movie of the formation of water-in-oil-in-water emulsion droplets for the production of artificial cell-mimics with hydrogel nuclei. Some inhomogeneity can be observed in droplet sizes and breakage of droplets that results in polymer beads after polymerization. Flow rates were 300  $\mu\text{l/h}$  for the outer aqueous, 40  $\mu\text{l/h}$  for the middle organic and 12  $\mu\text{l/h}$  for the inner aqueous phase.

### **Movie 2. Expression and capture of TetR-sfGFP in hydrogel nuclei of cell-mimics.**

Timelapse movie of the expression of TetR-sfGFP in cell-mimics containing pT7-*tetR*-sfGFP expression and 240x *tetO* array plasmids (see Fig. 1). Merge of brightfield channel and sfGFP fluorescence (green).

### **Movie 3. Communication between cell-mimics via a diffusive genetic activator signal.**

Activation of gene expression from T3 RNAP producing activator cell-mimics (magenta) to reporter cell-mimics (see Fig. 3). Merge of brightfield channel, sfGFP fluorescence (green) and rhodamine B fluorescence (magenta).

## **References**

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