Supporting Information

Artificial Gel-based Organelles for Spatial Organization of Cell-free Gene Expression Reactions

Lukas Aufinger, Friedrich C. Simmel*

Abstract: In biological cells, chemical processes often occur inside specialized subcellular compartments or organelles. For instance, in eukaryotes mRNA is transcribed and processed inside the nucleus, exported to the endoplasmic reticulum, and translated into the encoded protein. Inspired by this high degree of intracellular organization, we here develop gel-based artificial organelles that enable sequence-specific and programmable localization of cell-free transcription and translation reactions inside an artificial cellular system. To this end, we utilize agarose microgels covalently modified with DNA templates coding for various functions and encapsulate them into emulsion droplets. We show that RNA signals transcribed from transcription organelles can be specifically targeted to capture organelles via hybridization to the corresponding DNA addresses. We also demonstrate that mRNA molecules, produced from transcription organelles and controlled by toehold switch riboregulators, are only translated in translation organelles containing their cognate DNA triggers. Spatial confinement of transcription and translation in separate organelles is thus superficially similar to gene expression in eukaryotic cells. Combining communicating gel spheres with specialized functions opens up new possibilities for programming artificial cellular systems at the organelle level.

Table of Contents

1. Methods

1.1 Preparation of PITC-agarose

The reaction conditions were adapted from FITC protein labelling protocols, while the workup was inspired from the preparation of FITC labelled dextrans[1]. Typically, 1 mmol (306 mg) of super low melting (SLM) agarose (Carl Roth, #HP45.1) was dissolved in 10 mL of carbonate buffer pH 10 by heating to 90°C in a Falcon tube. The solution was cooled to room temperature and 0.25 mmol (22.9 µL) of propargyl-isothiocyanate (PITC, ChemPur Feinchemikalien und Forschungsbedarf GmbH, #FL-9569-1) dissolved in 5 mL of DMSO were added. After vortexing, the reaction was carried out for 12 hours at RT on a rotator.

The product was precipitated 4 times with 2 volumes (20 mL) of cold (-20°C) iso-propanol, washed with 1 volume of methanol, dried for 2 h in a desiccator and re-dissolved in 1 volume of ddH_2O. The first precipitation was carried out with 30 mL IPA and the final precipitate was dried overnight. The light brown flakes were stable for storage at 4°C for at least 9 months. Typical recovery was ~70-90%. The degree of substitution was assessed by reacting 50 µM of the fluorogenic dye 3-azido-7-hydroxycoumarine (Carl Roth, #7811.1) with 5x dilutions of PITC-agarose in unmodified SLM agarose, under the conditions shown in **Table S2**. The calibration curve (**Figure S1**) was obtained using propargyl-NHS (Sigma #764221) as an alkyne standard, because of its higher solubility in water compared to PITC.

1.2 Immobilization of DNA to PITC-agarose

Copper-catalyzed click reactions were performed in aqueous solutions under standard conditions[2]. Typically, ~10 mg of PITC-agarose were dissolved in ddH_2O to a concentration of 2% (w/v) assisted by heating at 80°C for 15 minutes and repeated vortexing and stirring, using round-bottom shaped 2 mL tubes. 100 mg (100 µL) of the viscous solution were pipetted to a fresh tube using a scale to improve precision and the reagents listed in **Table S2**, including azide-modified DNA were added. The solution was mixed thoroughly and the reaction was allowed to proceed for at least 2 hours at 45°C on a shaker at 350 rpm. Optionally, a small amount of pre-labelled PITC-agarose was added after DNA coupling, for barcoding. Depending on the reporter molecule, we used 3-azido-7-hydroxycoumarine (Carl Roth, #7811.1), 5-TAMRA-PEG3-Azide (baseclick GmbH, #BCFA-037), or Cyanine 5 azide (baceclick GmbH, #BCFA-082). Uncoupled DNA and the reaction buffer were removed through excessive washing after emulsion polymerization.

The final DNA concentrations are listed in **Table S5**. We note that for comparison of transcription and translation rates at different gel concentrations, the click reaction was performed in a master mix containing 1% PITC-agarose, which was then mixed with 4% PITC-agarose and 1x PBS to ensure an equal DNA concentration for all samples.

1.3 Emulsion Polymerization

After the click reaction the agarose solution was briefly heated at 80°C to ensure that the agarose is melted completely. The solution was centrifuged for 5 min at 16,000 g to remove any remaining debris that would interfere with droplet production. Then, 180µL of the supernatant were emulsified using a microfluidic flow focusing device (orifice width: 25 µm, height: 40 µm) for ca. 2 hours at RT. The pressure at the oil inlet was typically set to 390 mbar, while the aqueous phase was between 150 and 250 mbar, depending on the agarose density[1]. The droplet generation frequency was 1.5-2 kHz. The ca. 30 µm large functionalized microgel spheres were collected and gelled overnight at 4°C.

The emulsion was broken after removal of excess oil by addition of 1 volume (ca. 200 µL) of perfluoro-octanol (PFO, Alfa Aesar, #B20156.09) and 1 volume of 1x PBS, followed by gentle shaking. The mixture was spun down and the aqueous supernatant containing the microgel was transferred to a fresh tube. Finally, the microgel was washed 4 times with 1 mL PBS and 4 times with 1 mL nfH_2O by centrifugation for 1 minute at 2,000 rcf. The microgel was stored at 4°C and experiments were conducted within one month.

1.4 Encapsulation of Microgel Spheres

[1] The viscosity of dilute agarose solutions is in the order of 10 to 100 mPas. The viscosity of water is 1 mPas.

Gel organelles were encapsulated using a larger chip (orifice width: 40 µm, height: 60 µm), for < 30 minutes. Pressures were typically set to 150 and 70 mbar for the oil and aqueous phase, respectively. In order to delay transcription and cell-free protein expression (CFPE) reactions until data acquisition, the sample reservoirs and the collection tube were placed on ice. The monodispersity of gel organelles was crucial to ensure proper encapsulation without larger organelles clogging the channels.

1.5 Preparation and Design of DNA Templates

All DNA sequences are listed in **Table S1**. Modified oligos and templates for RNA aptamers were synthesized by IDT. The template for the dBroccoli (dB) aptamer was purchased as a gBlock, while the Malachite Green (MG) aptamer was PCR amplified from the coding strand purchased as 'Ultramer'. mVenus controlled by the toehold switch was a gift from Elisabeth Falgenhauer.

Templates were PCR amplified, using Phusion HF polymerase (NEB, #M0531), and purified with PCR cleanup columns (NEB, #T1030), or gel extraction (NEB, #T1020). Template concentrations were estimated by UV/Vis spectroscopy.

Linear DNA templates were by default flanked with standard primers (VF2 and VR). For immobilization we used a 5'-azide modified primer proximal to the T7 promoter pointing towards the free end. Consequently, transcribed RNA contained an extension complementary to the reverse primer at the 3' end which we used to capture RNA in microgel spheres equipped with 5'-azide modified reverse primer. The primer locations for the dB and MG templates were swapped to get orthogonal linker sequences. tr1 was immobilized at the 3' end hypothesizing that this orientation would ensure good accessibility of the RBS for ribosomes.

1.6 In Vitro Transcription and Cell-free Protein Expression Reactions

Transcription and translation reactions were prepared on an RNase free workbench on ice. The *in vitro* transcription (IVT) reactions contained transcription buffer (NEB, #B9012), rNTPs (NEB, #N0466), RNase Inhibitor Murine (NEB, #M0314), dense microgel suspension, T7 RNA polymerase (homemade, prepared by Dr. Sandra Sagredo) and salts as listed in **Table S3**. PURExpress (NEB, #E6800) was used with a final volume of 30 µL per reaction as described in **Table S4**.

1.7 Microfluidics

Photolithography

Photomasks (Zitzmann GmbH, 64.000 dpi) were designed in AutoCAD. All photolithography steps were performed in a cleanroom using 2-inch Silicon wafers (Siegert Wafer) and SU-8 2025 (micro resist technology), following the manufacturer's instructions, with parameters chosen as suggested for the desired resist thickness. The resist thickness was measured with a Dektak 150 surface profiler (Veeco instruments).

Soft-Lithography

12 g PDMS and curing agent (Sylgard 184, Dow Corning) were mixed thoroughly, poured on a clean silicon-master wrapped in aluminum foil, degassed for ≈ 15 minutes and baked for 75 minutes at 80°C. The cured PDMS devices were carefully disassembled from the master and trimmed with a scalpel. Inlet holes were punched with a 1.25 mm biopsy punch (WPI, #504530). Devices were sonicated in isopropanol for 15 minutes, rinsed with ddH2O and dried at 70°C. As substrate 2 × 3 inch object slides were coated with ≈ 5 g of PDMS to render the surface hydrophobic and minimize sticking of aqueous solutions.

Finally, substrate and device were bonded by exposure to O_2 plasma (30 seconds, 20 sccm, 100W). Bonding was completed at 80°C for 1 hour and hydrophobic recovery of PDMS was accelerated by baking at 200°C for 3 hours[3].

Device Operation

Microfluidic devices were operated with an Elveflow OB1 pressure controller. The continuous phase contained FC-40 oil (Sigma Aldrich, # F9755) with 2% (w/w) PFPE/PEG-surfactant[4] (Raindance Technologies). The aqueous phase typically contained 30 to 100 µL of transcription or transcription/translation mixture, or 180 µL of modified SLM agarose.

The sample reservoirs were connected to the chip by ca. 15 cm of inert PTFE tubing (BOLA, #S1810-10, OD 1.6 mm, ID 0.8 mm), directly plugged into the PDMS. Droplets were collected in an Eppendorf tube connected to the outlet via ca. 5 cm of PTFE tubing. The droplet generation was monitored with a microscope.

1.8 Epifluorescence Microscopy and Data Analysis

Microscopy Slides

Two cover glasses (60 mm × 24 mm and 40 mm × 24 mm) were first cleaned with a Kimwipe soaked in 2% Hellmanex® III, rinsed with ddH_2O and sonicated in ddH_20 for 5 minutes. The glass surface was rendered hydrophobic by wiping the slides with Kimwipes soaked in windshield water repellant (RainX). Remaining stains were removed by wiping the treated slides with iso-propanol and rinsing with ddH_2O. Finally, the slides were dried in an oven at 50°C.

To create channel walls with a defined height we mixed dentist glue (picodent twinsil® 22) with 75 µm glass beads (Sigma, #59200-U) acting as spacers. We then dipped the edge of an object slide into the mixture, imprint 2 thin lines spaced by ~15 mm on the large treated cover glass and apply the small cover glass.

Microscopy chambers were filled with ~20µL of emulsion, making sure that droplets are distributed homogeneously, by occasionally spacing the droplets with oil. The outlets were dried with Kimwipes and sealed with dentist glue.

Microscopy

Fluorescence time-lapse videos were recorded for up to 24 h at 37°C with a 10x P-Apo air objective (NA 0.45) on a Nikon Ti-2E, equipped with a SOLA SM II LED light source, a motorized stage, perfect focus system, an Andor NEO 5.5 camera and the filter sets listed in **Table S6**.

Typical settings were 50% brightness of the fluorescence LED and 500 ms exposure time. For measurements with dBroccoli we included a 20 second wait period prior to acquisition of the dBroccoli signal to avoid potential temporary bleaching of DFHBI-1T[5] by scattering light from close by positions.

Droplet Tracking

Bright field and fluorescence time-lapse videos were processed in ImageJ. First, all channels were binned 2x2. Fluorescence images were further flat field corrected to account for the inhomogeneous illumination profile. The images were background subtracted (ImageJ run("Subtract Background...", "rolling=100 stack");) and divided by appropriate flat field images obtained from a microscopy chamber filled with 100 nM fluorescein.

Images were then segmented based on the BF images using the Otsu method and tracked using a custom Matlab program that was previously developed in our lab[6] (https://github.com/kkapsner/Matlab.git), typically using the default settings. Prior to tracking regions with an area smaller than 500 px and an eccentricity[2] >0.6 were filtered out.

After tracking the droplet data was further filtered by radius and the length of the data vector. Droplets that 'jumped' due to movement, were in a different focal plane or had distorted fluorescence signals due to scattering caused by dirt, were removed manually. The extracted dataset contains an array with the size and the mean fluorescence intensity for each channel for each time point for each droplet.

Data Analysis

For reporter channels we used the total fluorescence intensity in a droplet, which is proportional to the number of fluorescent molecules, rather than a measure of concentration, because the total fluorescence intensity is independent on the size of the encapsulating droplet.

The main characteristic of the time traces is their initial reaction rate which was determined by fitting a line to the start slope (first 1-5 hours/1-2 hours). We generally observed that rates correlate strongly with the end levels, indicating that the reactions stop due to degradation or exhaustion of reaction components.

The label signals were processed similarly as the reporter signals. As labelled spheres move and the label bleaches, we obtain the label fluorescence intensity data by fitting a line to the first 10-30 data points and take the

[2] The eccentricity is the ratio of the distance between the foci of the ellipse that has the same second-moments as the region and its major axis length.

y-intersection as a measure for organelle content. Next we bin the label fluorescence intensity using the MATLAB function histcounts. Depending on the number of peaks (corresponding to droplets containing *n* organelles) that appear in the histogram, we fit a sum of Gaussians to the histcounts, with the constraint that the means have to be separated sufficiently. The minima between the Gaussians, are used as bounds to cluster the data. For the peak with the highest label intensity, the upper boundary was chosen as two times the standard deviation of the fitted Gaussian.

Finally, we used these bounds to cluster the time traces for droplets containing *n* organelles. Here error bars correspond to the standard deviation of reporter production rate within a cluster. x-error bars in rate vs. label plots correspond to the standard deviations of the Gaussian fits. We note that the number of droplets with *n* organelles roughly follows a Poisson distribution as would be expected for an encapsulation process with independent gel organelles.

2. Discussion of Experimental Uncertainties

We have observed that the reporter rates have a coefficient of variation (CV) of about 20%-30% for CFPE and 50% for IVT. In the following we discuss whether this noise originates from stochastic effects, measurement noise or variations due to sample preparation.

2.1 Stochastic Noise

The volume of a typical organelle with a radius of 12.5 µm is ~8 pL. The concentrations of molecules are 10-100 nM for DNA, 100 nM or 250 nM for T7 RNA polymerase in PURE or transcription reactions, respectively, and 2,000 nM for ribosomes in PURE[7]. To be conservative, we assume a concentration of 1 nM. Then there are *N*=5,000 molecules in one organelle and the variations due to stochasticity would be $\frac{1}{\sqrt{N}} \approx 1.4\%$, which cannot explain the variations observed in the experiment.

2.2 Measurement Uncertainties

In Epifluorescence microscopy, fluorescence images are acquired with an inhomogeneous illumination profile which in our case has a CV of 22.1%. We therefore perform a flat field correction on the fluorescence images to correct for the inhomogeneous illumination. This usually reduces the CV to <5%. An additional source of variation that only applies to the label signals is that the organelles may be located in different focal plains within the droplet, reducing their overall brightness.

The bright field images that are used for segmentation display bright droplets surrounded by a dark edge. For the segmentation to work reliably the edge of the droplet needs to have a thickness of at least 1 pixel. This may explain a systematic uncertainty of <8% in droplet area for a droplet with a radius of 25 pixels. However, this should not lead to an increased heterogeneity in the measured signals when the droplet size distribution is narrow.

2.3 Uncertainties Related to Sample Preparation

We therefore conclude that the main source of variations is likely related to the sample preparation, which includes preparation of organelles, encapsulation in droplets and the reaction mixtures.

One batch of organelles is prepared from 180 µL of DNA modified agarose solution that is vigorously stirred prior to emulsification to ensure homogeneous distribution of DNA and fluorescent label. One possible source of variations is an inhomogeneous organelle radius which has a typical CV of <10%. This uncertainty would propagate to an uncertainty in the organelle volume of $\frac{\Delta V}{V} = \sqrt{\left(\frac{1}{V}\frac{\partial V}{\partial r}\Delta r\right)^2} = 3\frac{\Delta r}{r} = 30\%$. As the organelle volume is proportional to the number of DNA templates and we observe a constant reaction rate (within 24 hours), the uncertainty in organelle volume should ultimately propagate to an uncertainty in the observed reaction rate. However, the organelle volume should as well correlate with the label intensity which we do not observe.

Uncertainties can also be caused during the encapsulation of organelles in droplets. Firstly, also droplets have a varying radius, typically with a similar CV of <10%. However, as one organelle produces a constant amount of RNA or protein, this is corrected for by taking the total fluorescence intensity in a droplet rather than a measure proportional to concentration.

While general batch to batch variations and pipetting errors in the reaction mix can only explain cross experiment variations and not variations between droplets of the same set, one possible effect to consider is adsorption of

reactants like T7 RNA polymerase to PDMS and PTFE surfaces. This would mean that droplets that are generated early contain less functional enzymes and therefore exhibit lower reaction rates. One aspect that supports this thesis is that the observed CVs were consistently higher for IVT than in PURE, which overall contains more macromolecules and crowding agents. A related source of uncertainties is that, despite cooling, reactions may occur in the solution prior to encapsulation, as observed in **Figure 4** and **Figure S5**, causing enhanced background signals.

In summary, the observed uncertainties are most likely caused by variations in sample preparation. Further improvement of organelle monodispersity and the encapsulation procedure itself may be the most promising candidates to reduce the experimental uncertainty.

3. References

[1] A. N. De Belder, K. Granath, *Carbohydrate Research* **1973**, *30*, 375-378.
[2] A. E. Speers, B. F. Cravatt, *Chemistry & biology* **2004**, *11*, 535-546.
[3] S. Kaneda, K. Ono, T. Fukuba, T. Nojima, T. Yamamoto, T. Fujii, *Analytical Sciences* **2012**, *28*, 39.
[4] C. Holtze, A. C. Rowat, J. J. Agresti, J. B. Hutchison, F. E. Angilè, C. H. J. Schmitz, S. Köster, H. Duan, K. J. Humphry, R. A. Scanga, J. S. Johnson, D. Pisignano, D. A. Weitz, *Lab on a Chip* **2008**, *8*, 1632.
[5] P. Wang, J. Querard, S. Maurin, S. S. Nath, T. Le Saux, A. Gautier, L. Jullien, *Chemical Science* **2013**, *4*, 2865.
[6] K. Kapsner, F. C. Simmel, *ACS Synthetic Biology* **2015**, *4*, 1136-1143.
[7] Y. Shimizu, T. Kanamori, T. Ueda, *Methods* **2005**, *36*, 299-304.

4. Supplementary Figures

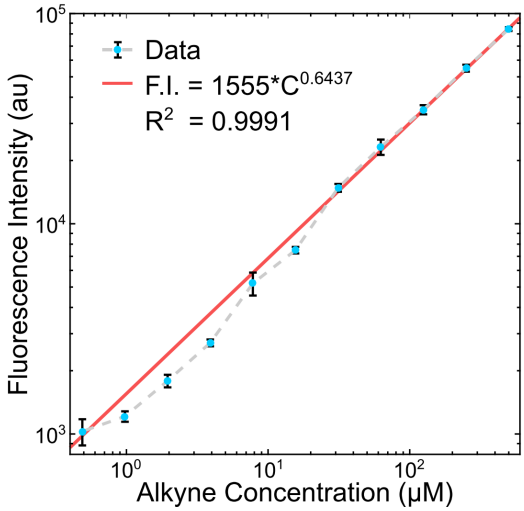

Figure S1. Calibration curve for estimation of the gel functionalization efficiency using 3-azido-7-hydroxycoumarine. The data was fitted with a power law. Error bars represent the standard deviation of three technical replicates.

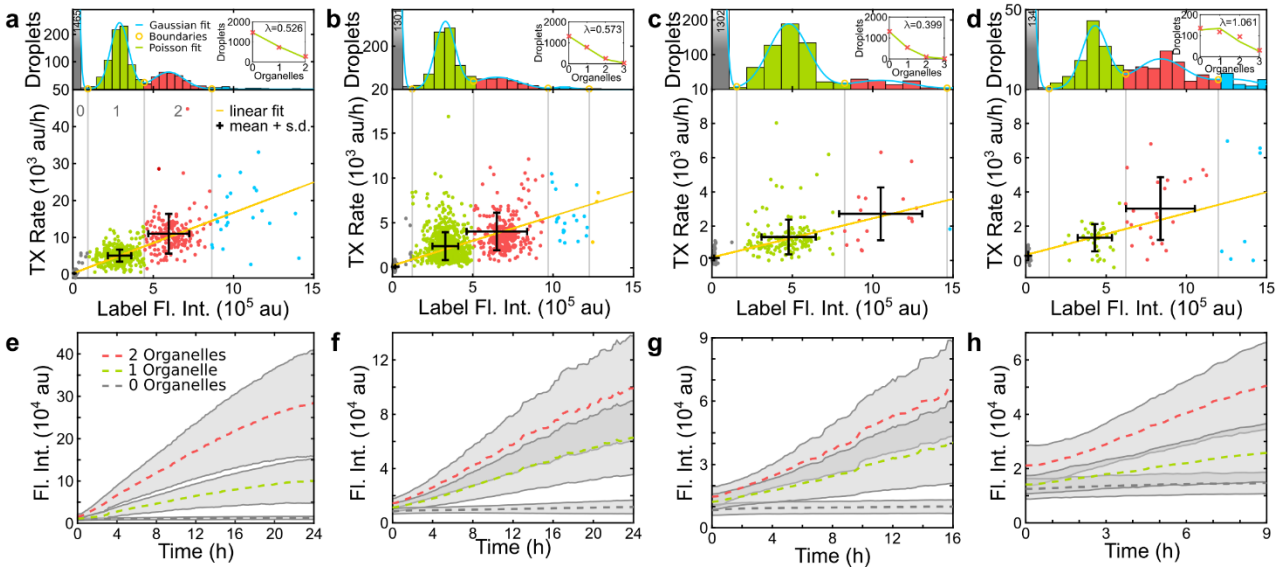

Figure S2. Full data set corresponding to Figure 2d. **a)-d)** Scatter plots of the transcription rate versus the organelle label intensity along with the respective histogram used for classification of the data. **e)-h)** Average time traces for droplets with equal organelle content. Shaded areas are standard deviations. The agarose densities were **a), e)** 0.66%, **b), f)** 1.0%, **c), g)** 1.5% and **d), h)** 2.25%. The time traces for 1.5% and 2.25% were partly corrupted due to droplet movement and are therefore shown for a shorter time span. However, as the transcription rate was fitted between hours 1 to 5, the set was still considered valid.

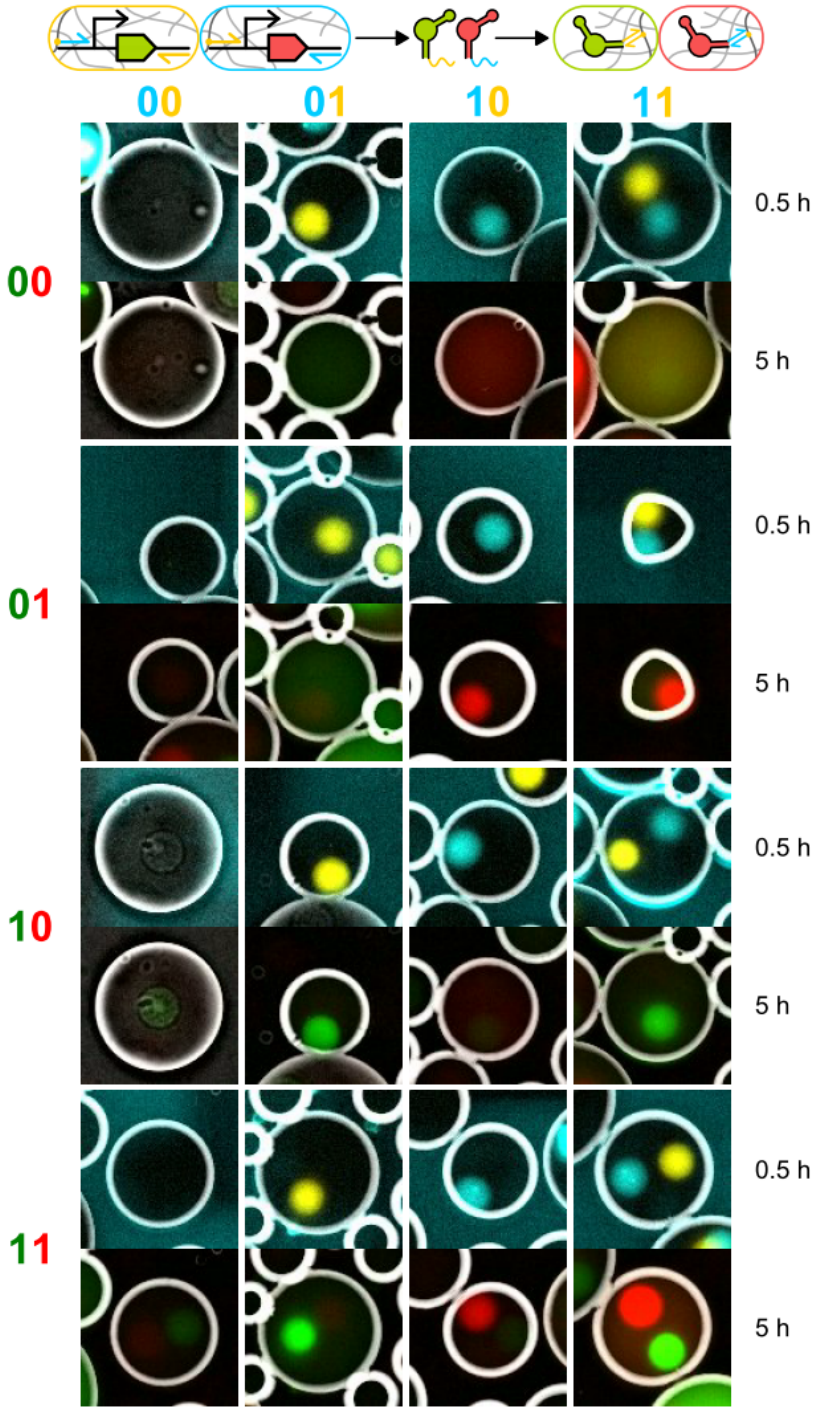

Figure S3. All binary combinations of transcription and capture organelles. Droplets with equal content of transcription organelles are ordered in columns, while capture spheres are ordered in rows as indicated. The channels for transcription organelles (yellow, cyan) and RNA reporters (green, red) are shown separately for clarity. Following, e.g., the 2nd column (01), it is clear that in all cases the green dBroccoli aptamer is produced, but not the red Malachite Green. The RNA is localized in presence of the respective capture sphere (row 10 and 11) and delocalized otherwise. The lower rightmost droplet (11/11) is the same as in Figure 2e.

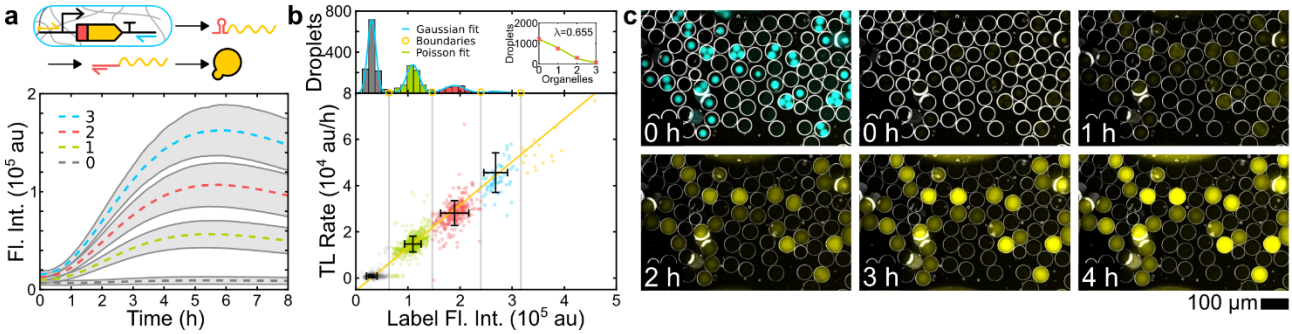

Figure S4. Gene expression from transcription organelles containing th1-mVenus DNA with tr1 in solution. **a)** Average time traces of droplets with equal organelle content, obtained from the histogram in b). **b)** Scatter plot of the expression rate versus the organelle label intensity. All error bars and shaded areas indicate standard deviations. **c)** Exemplary time series showing the expression of mVenus (yellow) in droplets containing transcription organelles (cyan, top left).

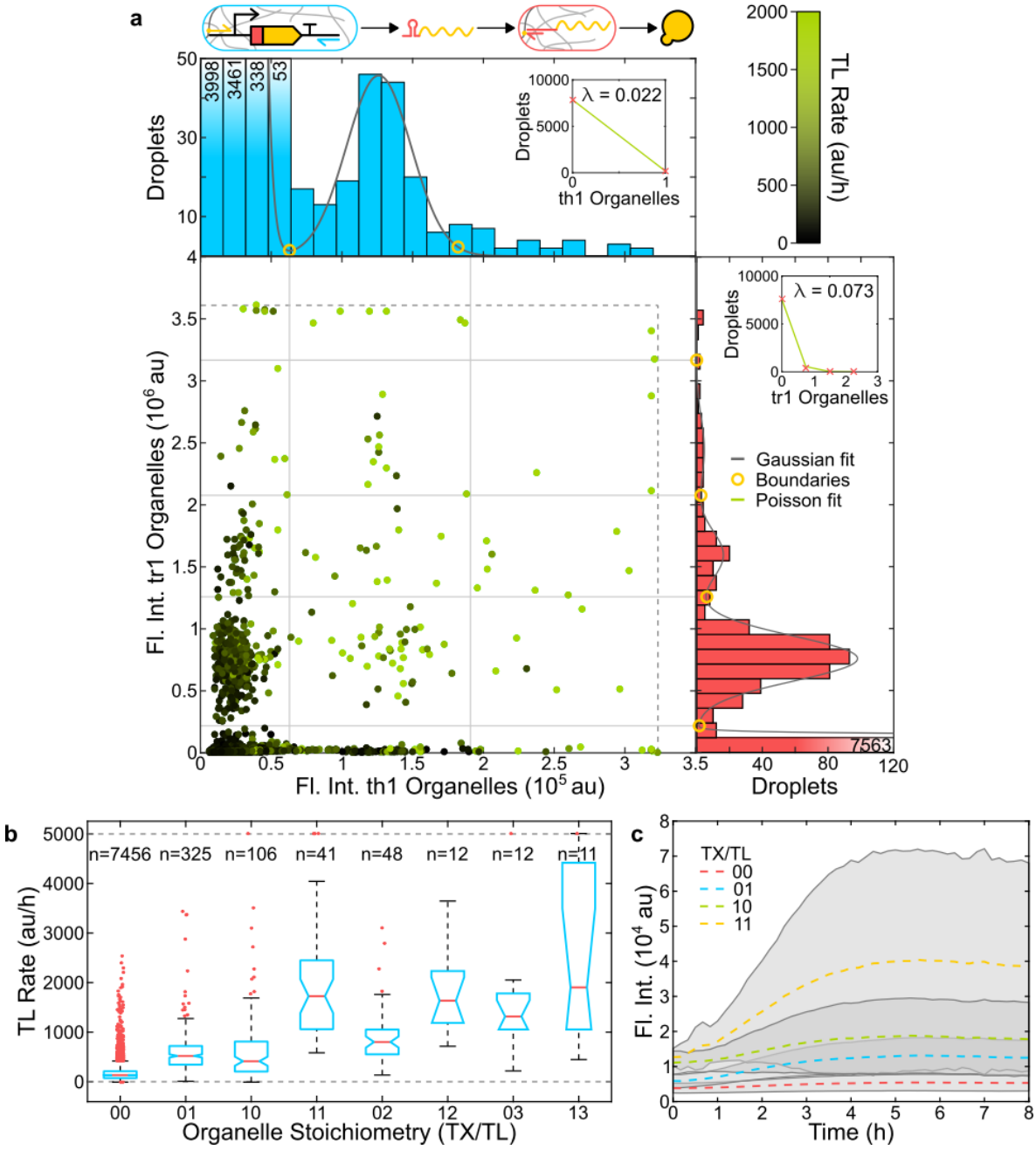

Figure S5. Supporting data for the combination of transcription and translation organelles corresponding to Figure 4. **a)** Scatter plot of the label intensities of transcription and translation organelles. The green color indicates the translation rate in the droplet. Histograms were used to divide the data into populations as indicated by the gray lines. The label intensity data was cropped at the 99.9% percentile (dashed lines). **b)** Extended boxplot including the data with multiple translation organelles. **c)** Average time traces for different organelle stoichiometry. Shaded areas represent standard deviations.

5. Supplementary Tables

Table S1. List of DNA sequences, transcripts in capital. Yellow/blue: primer binding sites, red: trigger sequence, purple: T7 promoter, green: T7 terminator BBa_B10015.

Name	Sequence
VF2	/5AzideN/tgccacctgacgtctaagaa
VR	/5AzideN/attaccgcctttgagtgagc
tr1	gcagggataaacgagatagataagataagatag/3AzideN/
dBroccoli (dB)	tgccacctgacgtctaagaaccttaatacgactcactataGGGAGAAGCCTGAGACGGTCG GGTCCATCTGAGACGGTCGGGTCCAGATATTCGTATCTGTCGAGTAGAG TGTGGGCTCAGATGTCGAGTAGAGTGTGGGCTCAGGCTTCTCCCGCTCA CTCAAAGGCGGTAAT
Malachite green (MG)	attaccgcctttgagtgagccttaatacgactcactataGGGAGACTGGATCCCGACTGGC GAGAGCCAGGTAACGAATGGATCCAGTCTCCCTTCTTAGACGTCAGGTG GCA
th1-mVenus	tgccacctgacgtctaagaaaaggaatattcagcaatttgcccgtgccgaagaaaggcccacccgtgaag gtgagccagtgagttgattgctacgtaattagttagttagcccttagtgactcgaattctaatacgactcactata GGGTCTTATCTTATCTATCTCGTTTATCCCTGCATACAGAAACAGAGGAG ATACGCAATGATAAACGAGAACCTGGCGGCAGCGCAAAAGAGCAAAGGC GAAGAACTGTTCACGGGTGTGGTTCCGATCCTGGTTGAACTGGATGGCG ATGTGAACGGTCATAAATTTAGCGTGTCTGGTGAAGGCGAAGGTGATGC GACCTACGGCAAACTGACGCTGAAACTGATTTGCACCACGGGTAAACTG CCGGTTCCGTGGCCGACCCTGGTGACCACGCTGGGTTATGGTCTGATGT GTTTCGCACGTTACCCGGATCACATGAAACGCCATGATTTCTTTAAATCT GCGATGCCGGAAGGCTATGTGCAGGAACGTACCATCTTTTTCAAAGATG ATGGTAACTACAAAACCCGCGCGGAAGTTAAATTTGAAGGCGATACGCT GGTGAACCGTATTGAACTGAAAGGTATCGATTTCAAAGAAGATGGCAATA TTCTGGGTCACAAACTGGAATACAACTACAACAGTCATAACGTGTACATT ACCGCCGATAAACAGAAAAACGGTATCAAAGCAAACTTCAAAATCCGTCA CAACATCGAAGATGGCGGTGTTCAGCTGGCCGATCATTACCAGCAGAAC ACCCCGATTGGCGATGGTCCGGTGCTGCTGCCGGATAATCATTATCTGA GTTACCAGAGCAAACTGTCTAAAGATCCGAATGAAAAACGCGATCACATG GTTCTGCTGGAATTTGTGACCGCGGCCGGCATTACGCATGGTATGGATG AACTGTATAAATAACCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAG ACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAG AGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTATAactagtagc ggccgctgcaggagtcactaagggttagttagttagattagcagaaagtcaaaagcctccgaccggaggc ttttgactaaaacttcccttggggttatcattggggctcactcaaaggcggtaat

Table S2. Standard conditions for copper-catalyzed click reactions.

Reagent	Volume (µL)	Stock concentration	Final concentration
PITC-agarose	100	2% (= 50 µM alkyne)	1% (= 25 µM alkyne)
PBS	40	5x	1x

Azide-DNA	-	0.3 µM – 0.5 mM	50 nM – 10 µM
ddH_2O	to 200		
TCEP	2	100 mM	1 mM
THPTA	4	5 mM	0.1 mM
$CuSO_4$	2	100 mM	1 mM
Labelled agarose (opt.)	4	50 µM dye	1 µM dye

Table S3. Conditions for IVT reactions.

Reagent	Volume (µL)	Stock concentration	Final concentration
nfH_2O	to 100		
NEB transcription buffer	10	10x	1x
$MgCl_2$	6	200 mM	12 mM
KCl	10	1,250 mM	125 mM
rNTP (each)	16	25 mM	4 mM
RNase Inh. Murine	2.5	40 U/µL	1 U/µL
Gel organelles	≤40		
T7 RNAP	2.5	10 µM	0.25 µM

Table S4. Conditions for CFPE reactions.

Reagent	Volume (µL)	Stock concentration	Final concentration
Solution A	12		
Solution B	9		
RNase Inh. Murine	0.75	40 U/µL	1 U/µL
nfH20	to 30		
Free DNA (opt.)	≤8		
Gel organelles	≤8		

Table S5. Overview of organelle composition in each experiment.

Figure	DNA	DNA concentration	Gel concentration
2a-d/S2	dB	50 nM	0.66%-2.25%
2e/S3	dB/MG	150 nM	1%
2e/S3	VF2/VR	10 µM	1%
3	tr1	1 µM	1%

4/S5	th1-mV	60 nM	1%
4/S5	tr1	1 µM	0.66%
S4	th1	60 nM	1%
S4	tr1	2 µM	free

Table S6. Filters used in Epifluorescence microscopy.

Dye	**Dichroic (nm)**	$\boldsymbol{\lambda_{ex}}$ **(nm)**	$\boldsymbol{\lambda_{em}}$ **(nm)**
coumarin	458	438/24	483/32
dBroccoli	495	472/30	520/35
TAMRA/ mVenus	550	532/10	585/64
unused	585	559/34	639/69
Cy5/ Malachite Green	660	628/40	692/40