Electronic Supplementary Material (ESI) for Lab on a Chip.

DNA-functionalized hydrogels for confined membrane-free in vitro transcription/translation

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Supporting Information

Cloning of pRSET-deGFP vector: Both DNA constructs, deGFP and GFP-His, are based on the pRSET vector, and all cis-elements of the transcription control are identical, as checked by Sanger sequencing, merely the coding sequence for deGFP was replaced by the coding sequence for GFP-His (73 % accordance on coding sequence level, 97 % accordance on protein level). The vector acting as target for PCR amplification was produced by combining the deGFP coding sequence (CDS) of pBEST-OR2-OR1-Pr-UTR1-deGFP-T500 plasmid (kindly provided by Prof. Dr. Vincent Noireaux, University of Minnesota, USA) with the vector backbone of a pRSET5d-GFPHis plasmid. The deGFP CDS was PCR amplified with primers that introduced a *Nco*I restriction site at the 5'end of the CDS and a *Xho*I restriction site at the 3'end. Both the PCR product and the pRSET5d-GFPHis vector were double-digested with *Nco*I and *Xho*I, the individual bands of desired size were excised and purified using a QIAquick Gel Extraction Kit (Qiagen, Germany). The resulting fragments were ligated using T4 DNA ligase (New England Biolabs, USA). The ligation mix was transformed with a standard heat shock protocol into *E.coli* XL-1 blue cells. After antibiotic selection, a single colony was picked, grown in a 5 mL-LB-medium culture and the plasmid DNA was isolated using a QIAprep Spin Miniprep Kit (Qiagen, Germany). The clone was tested via

restriction digestion/gel electrophoresis and sequencing analysis (GATC Biotech, Germany). The PCR used the two deGFP specific 5'-TATA**CCATGG**AGCTTTTCACTGGC-3' (10-MH-pBEST-eGFP 5') and 5'-GTAC**GAATTC**TTGCTCGAGTTAGATCC-3' (11-MH-pBEST-eGFP 3') where the orange sequence indicates the ***Nco*I** restriction site, blue the ***Xho*I** restriction site and bold the sequence specific part. DNA sequencing of the insert in the pRSET5d-deGFP vector was done with 5'-TAATACGACTCACTATAGGG-3' (1-DF-T7 forward primer (Promoter)) and 5'-GCTAGTTATTGCTCAGCGG-3' (2-DF-T7 reverse primer (Terminator)).

Polymerase chain reaction (PCR): PCR reactions were done in a T Professional Thermocycler (Biometra) in 50 µL or 100 µL reactions. PCR reactions were done under following conditions unless otherwise specified. Primers were purchased from Integrated DNA Technologies (IDT®, USA). Each reactions contained in final concentrations 20 mM Tris-HCl (pH 7.9), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 20 mM $MgSO_4$, 0.2 mM dNTPs, 1 µM forward primer, 1 µM reversed primer, 100 ng pRSET5d-deGFP DNA and 200 ng *Pfu* DNA polymerase. *Pfu* DNA polymerase was kindly provided by Frank Nelissen (Radboud University Nijmegen).[1] The reaction was initially incubated for 2 minutes at 95°C followed by 30 cycles of 30 seconds at 95°C, 45 seconds at 53°C and 4 minutes at 72°C. After the cycled steps all incomplete fragments were elongated at 72°C for 10 minutes. Afterwards the reaction was stored in 4°C (short time) or -20°C until purification using a QIAquick PCR Purification Kit (Qiagen, Germany). All PCR reactions were checked using Agarose gel electrophoresis. DNA concentrations were usually around 350-400 ng μL^{-1} measured by a NanoDrop Spectrophotometer ND-1000 (Thermo Scientific, USA).

The primers used to create the different DNA template fragments are listed in **Table 1**:

Primer name	Mod	Sequence 5'-3'
3-DF-pRSET5D GFP For		TTTTTGTGATGCTCGTCAGG
4-DF-pRSET5D GFP rev 5'Acrydite	5'Acrydite	AGGGAAGAAAGCGAAAGGAG
5-DF-pRSET5D GFP rev		AGGGAAGAAAGCGAAAGGAG
6-DF-pRSET5D AmpR out right		AGCCCTCCCGTATCGTAGTT
7-DF-pRSET5D AmpR out left		ATAATACCGCGCCACATAGC
8-DF-pRSET5D Ori		GCCTACATACCTCGCTCTGC
9-DF-pRSET5D GFP rev 5'I-Link	5'I-linker	AGGGAAGAAAGCGAAAGGAG
62-DF-primer 3 Alexa 647	Alexa 647	TTTTTGTGATGCTCGTCAGG

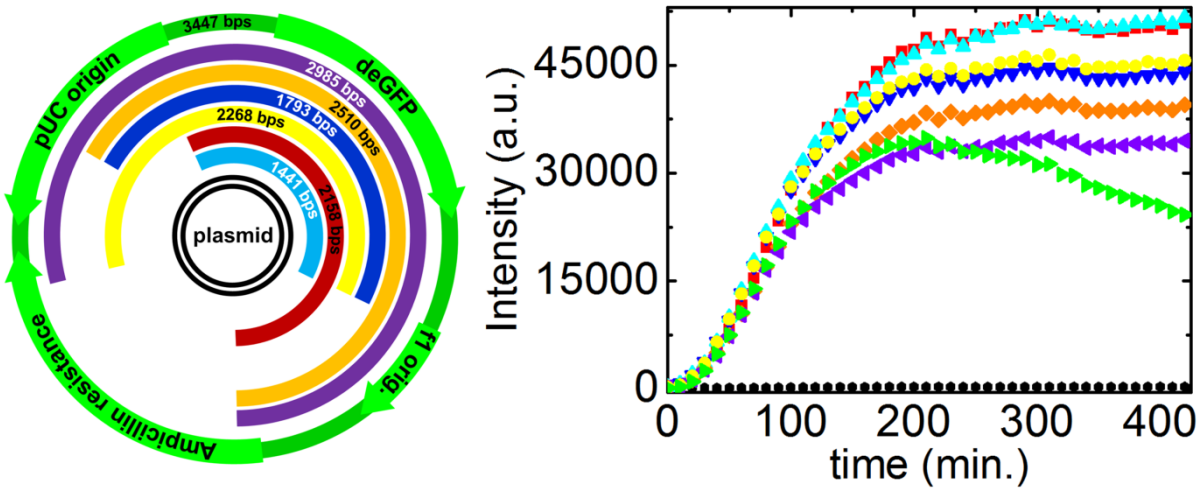

Figure S1: Design of a DNA templates for spatially localized gene expression that can be covalently attached to a hydrogel matrix. DNA fragments yielded by PCR employing different pairs of primers that bind to pRSET5d-deGFP plasmid DNA (left), deGFP expression of the corresponding DNA fragments (4 nmol per experiment) using a commercial IVTT kit in solution (right). Color coding of expression curves relates to the DNA fragments left, the background signal (black) is recorded from an IVTT kit (50 µL) without addition of DNA. 33,000 arbitrary units are equivalent to 1 µM of eGFP in a 50 µL IVTT reaction in a 96-well plate, determined on a Tecan Infinite 200 Pro plate reader.

The primer combination 3 and 5 yielded the same 1,441 bps large fragment which was used in this study, but then with primers 3 and 4 (bearing the Acrydite™ modification). Reverse primer 4 contains a 5' Acrydite™ modification and binds ~180 bps downstream of the transcription terminator site inside of the f1 origin of replication while the reverse primers (either blank (primer 3) or Alexa 647 (primer 62) labeled) binds ~300 bps upstream of the transcription start inside of the pUC origin of replication yielding a 1,441 bps long double stranded PCR fragment of which the template strand can be covalently attached to free thiol-groups present in the hydrogel particles (Figure 1). The test row shown in Figure S1 was conducted with identical primers not bearing the 5' modification. The modifications had no influence on bulk gene expression (data not shown).

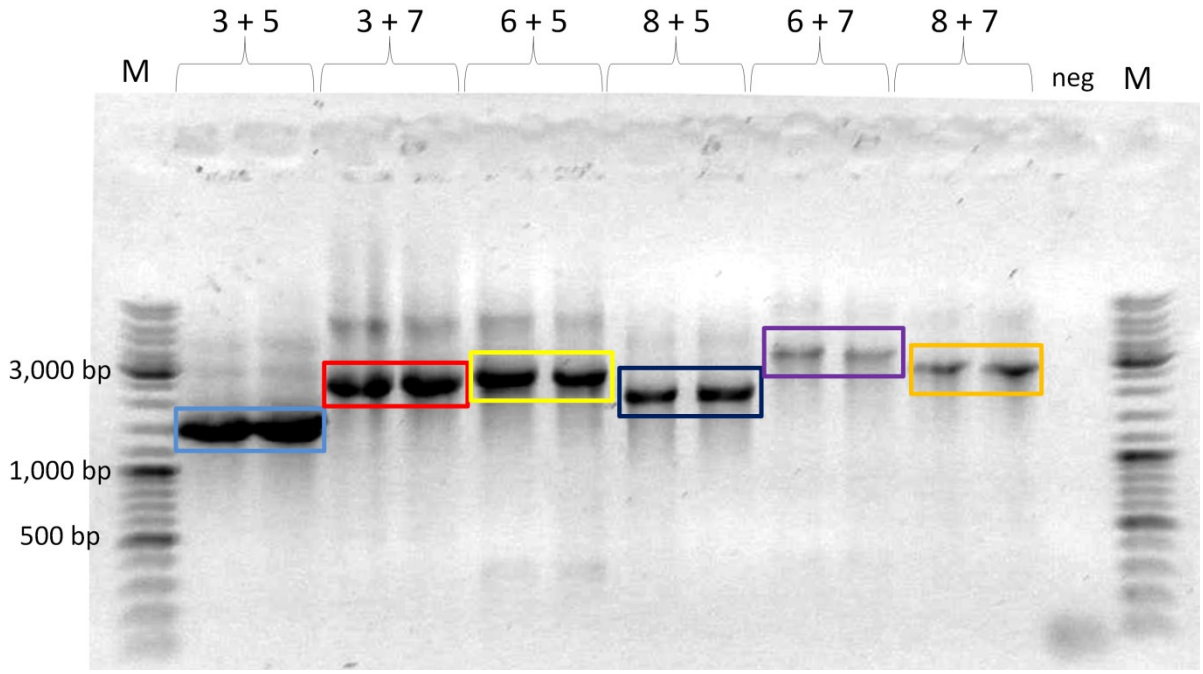

Figure S2: Ethidium bromide staining of an agarose gel. The number pairs above indicate the primers used for each PCR, which was conducted in duplicate. The colored frame indicates the main product band while the color is matched to the color-code in Figure S1. The faint bands above the target products are common artifacts in PCR and likely originate from nonspecific interactions during the PCR. "Neg" refers to a mock control for the PCR reaction.

Isolation and labeling of 70S Ribosomes for diffusion studies:

The protocol for low-level, non-specific labeling of ribosomes was adapted from Blanchard and colleagues.[2] The reaction was conducted in 50 mM Tris-HCl pH 7.6, 15 mM MgCl2, 100 mM NH_4Cl, 6 mM ß-mercaptoethanol. 5 µM ribosomes were incubated with 100 µM NHS-Alexa 647. Excess of free dye was subsequently washed using the same buffer and centricon 3000 spin columns (Merck Millipore, USA) and concentrated using Vivaspin 4 spin columns (Sartorius). The labeled ribosome concentration was estimated 2 µM with less than 30 % labeled with fluorescent dye.

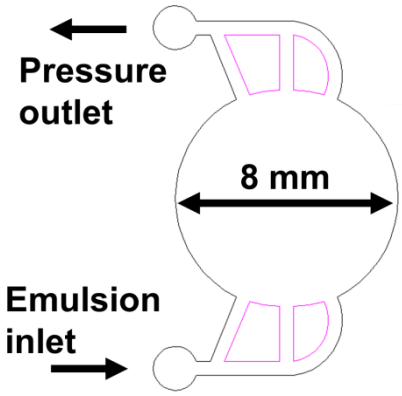

Figure S3: CAD-based drawing of a PDMS-based microfluidic chamber for storing water-in-oil emulsion droplets under static conditions suitable for long-term imaging.

Quantification of deGFP production from DNA-functionalized hydrogel particles

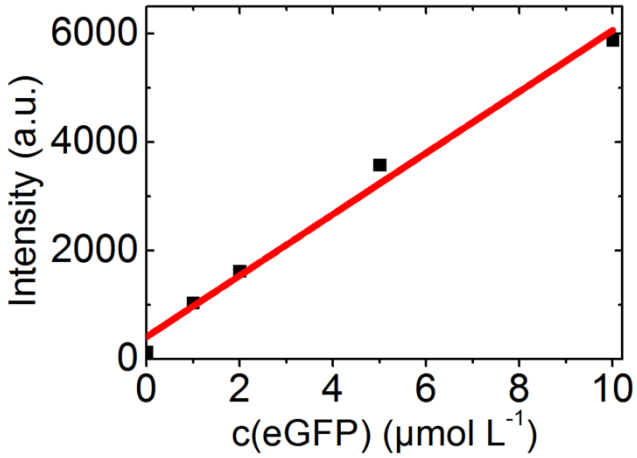

Figure S4: Calibration curve of four different eGFP concentrations and water.

To relate the fluorescence intensity given as arbitrary units from confocal microscopy experiments to the amount of deGFP produced in hydrogel particle-loaded IVTT microdroplets, defined amounts of eGFP were compartmentalized into water-in-oil droplets and imaged with the same settings on the confocal microscope as the experiments described in Figure 4 of the manuscript.

References

1. F. H. Nelissen, F. C. Girard, M. Tessari, H. A. Heus and S. S. Wijmenga, *Nucleic Acids Res*, 2009, **37**, e114.

2. S. C. Blanchard, H. D. Kim, R. L. Gonzalez, Jr., J. D. Puglisi and S. Chu, *Proc Natl Acad Sci U S A*, 2004, **101**, 12893.