Supporting Information for

Ultra-high density protein spots achieved by on chip digitalized protein synthesis

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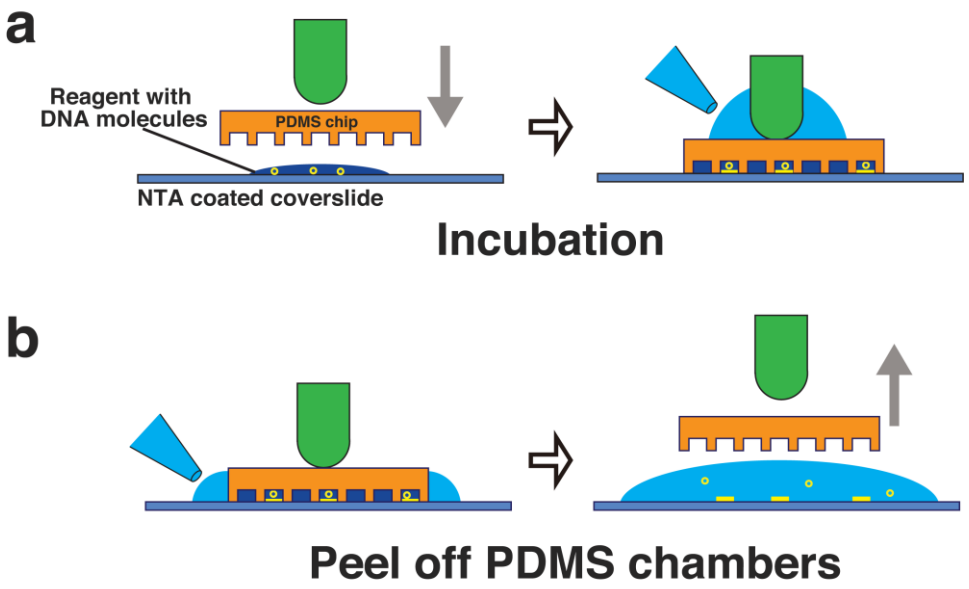

Figure S1. d-CFPS procedure. a) Incubation. A PDMS chip was prepared as described and removed from soaking water. CFPS lysate was prepared according to the manufacturer's instructions (Roche) with addition of Superase-In and template DNA. The mixture was prepared on ice. A drop of CFPS system (2 μL) was placed on the microscope coverslide and sandwiched with the PDMS sheet. Microchambers were sealed by pressing the PDMS sheet against the coverslide with a plastic tube controlled by a microactuator (Fig. S2). In order to prevent evaporation during the incubation period, a drop of water was regularly positioned above the PDMS sheet. The device was incubated at 30 °C for several hours with continuous fluorescence monitoring with an exposure time 1.6 s every 2 min. b) Peel off PDMS chambers. Curtain volume (200 μL) of PBS buffer with 0.05% Tween-20 was added between the gap of PDMS and Ni-NTA coated slides in order to dilute the cell free protein synthesis system and unbound proteins after peeling off PDMS chambers. The plastic tube was gradually removed. The PDMS sheet was separated from the microscope coverslide. The buffer on the slide was removed and new buffer was added to wash the slide 3 times. The protein capture slide was observed for fluorescence.

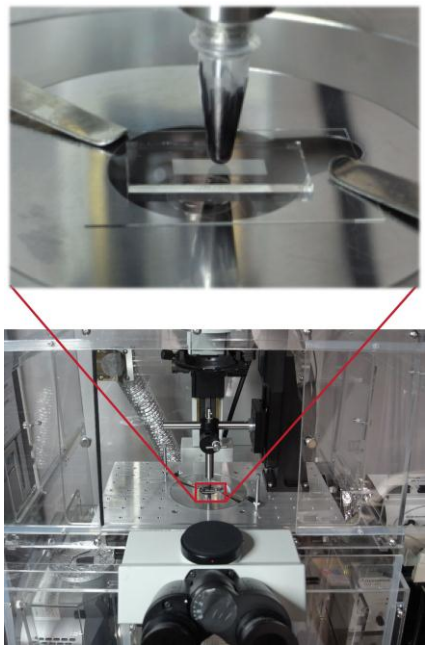

Figure S2. Experimental setup. The PDMS microchamber array was observed on an inverted microscope (IX 71, Olympus). To enclose the microchamber array, the PDMS chip is pressed on the microscope slide with rounded plastic tips connected to a motorized stage (SGAM20; Sigma koki co. Ltd.), controlled with a stage controller (SHOT-202AM; Sigma koki co. Ltd.). Temperature of the device was kept at 30 degrees during incubation inside of a temperature control box.

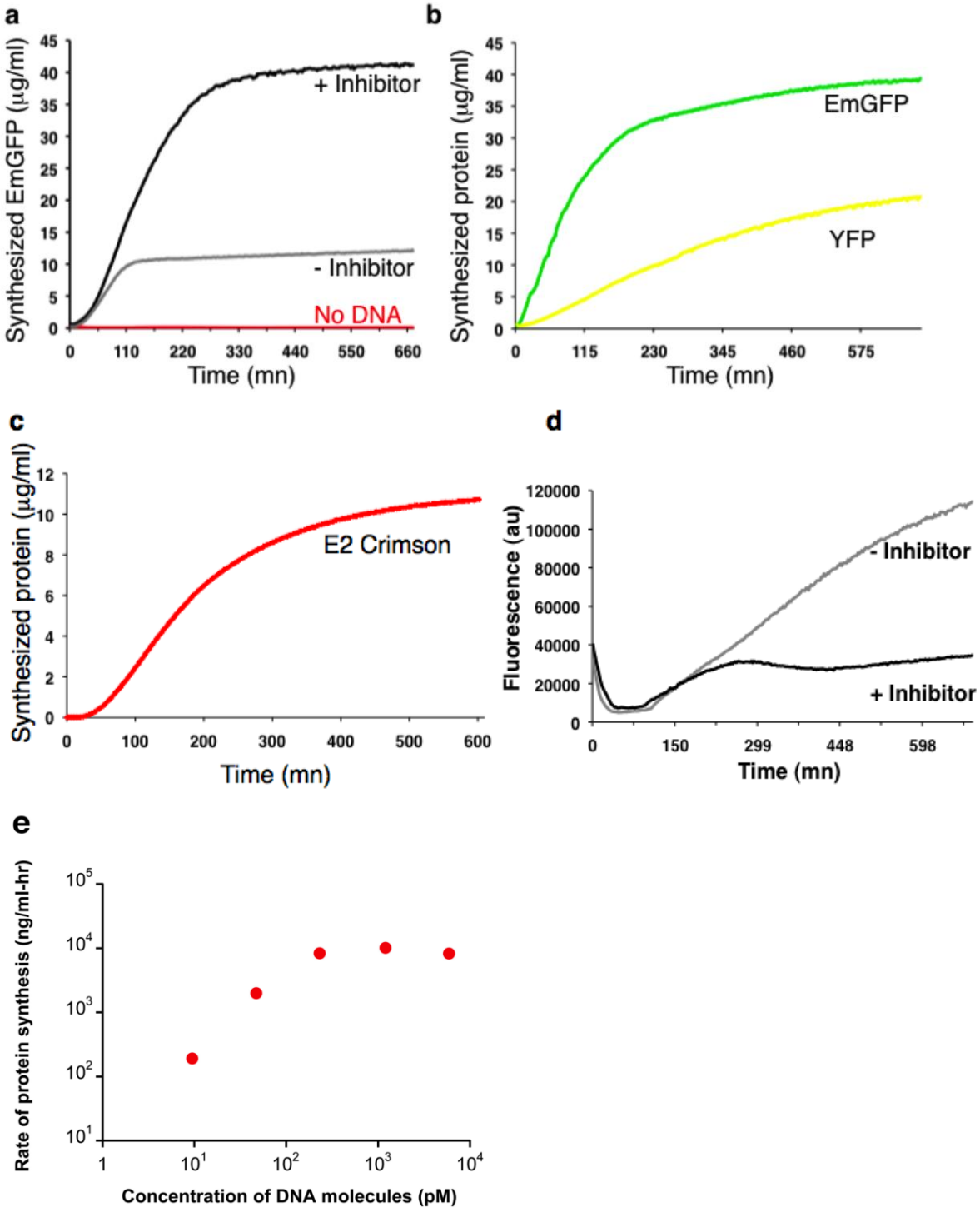

Figure S3. Optimization of CFPS performance. Optimization of protein synthesis efficiency and yield. Bulk assays were used to define the best CFPS system and components in order to maximize signal to noise ratio in d-CFPS experiments. Several fluorescent proteins were tested to look for optimal yields and fluorescence signal. a) Contamination with RNAses can lead to a decrease of CFPS yields. The use of RNAse inhibitor leads to an extension of mRNA lifetime during CFPS in RTS100 system (Roche) with a strong gain in the system

performance at 30°C. b) YFP synthesis shows slower kinetics compared to EmGFP; Ext 485 nm, Em 535 nm. No correction was applied to take into account slight differences in brightness and excitation/emission wavelengths. c) Synthesis of E2-Crimson. Differences in kinetics were observed between EmGFP, YFP and E2-Crimson, where the fastest to reach the plateau being EmGFP. d) During the course of protein synthesis without any template DNA, fluorescence background slightly increases. The RNA inhibitor maintains the fluorescence background to low levels. e) Total rate of EmGFP synthesis is linear with the concentration of DNA up to 150 pM. At saturating concentration of DNA, the rate of EmGFP synthesis is 10 mg/ml-hr at 30°C, in the same range as the value found for CFPS of GFP.[1,2]

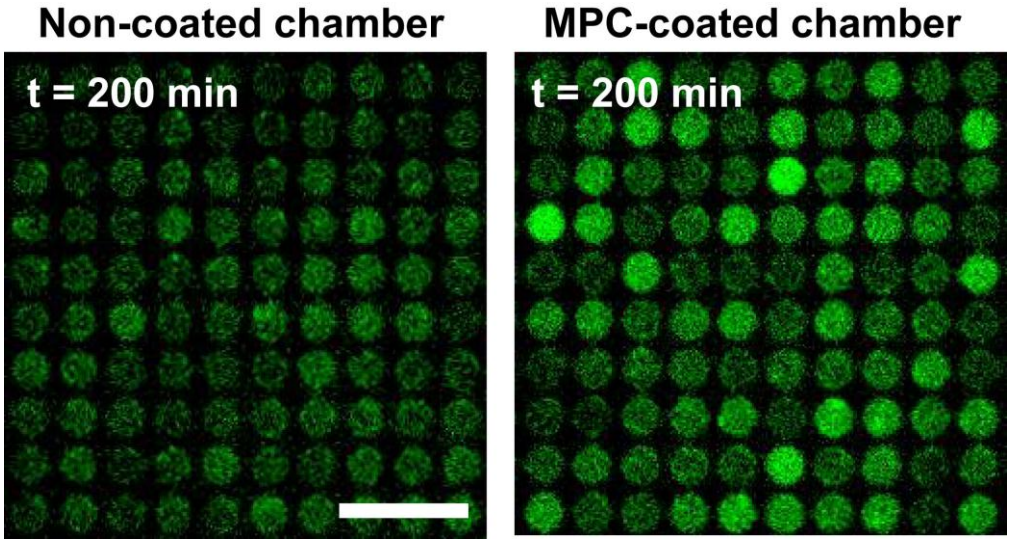

Figure S4. Biocompatibility surface treatment. Comparison of fluorescence levels for non-coated (left) and MPC-coated (right) PDMS chips. Although PDMS is widely used for micro fabrication, non-specific binding of biomolecules on PDMS surface needs to be overcomed to achieve true biocompatibility. In order to check the effect of MPC coating on CFPS, fluorescence intensities of EmGFP synthesized inside naked PDMS and MPC-coated PDMS chambers were compared. Same concentration of DNA molecules (1.2 molecules per chamber) was used for on-chip incubation with CFPS system (RTS 100 *E. coli* HY kit) for 200 min. MPC coating improved the yield of fluorescence signal of EmGFP by a factor 3. Scale bar is 30 μm.

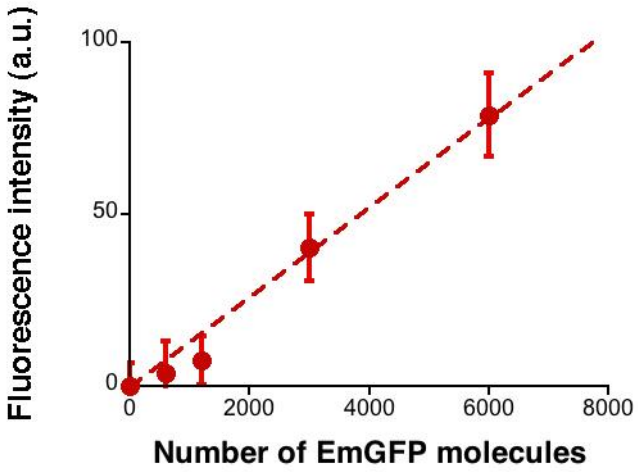

Figure S5. Calibration of EmGFP concentration in microchambers. Fluorescence intensities are plotted in function of the number of EmGFP molecules in the microchambers. EmGFP molecules were diluted in the CFPS system (RTS 100 *E. coli* HY kit) and trapped into MPC coated microchambers. Fluorescence images were recorded with identical setup as for d-CFPS experiments.

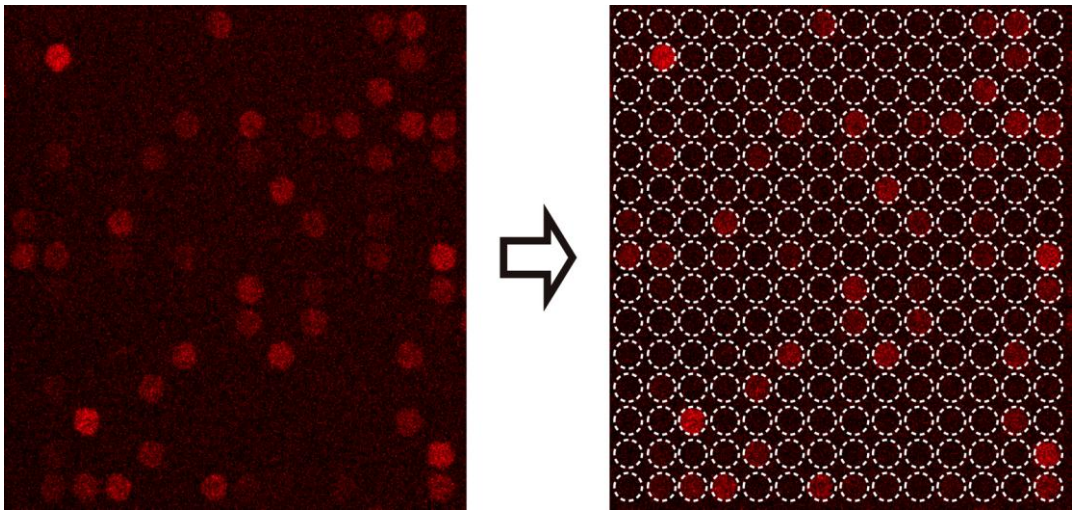

Figure S6. Analysis of E2-Crimson on the NTA coated slide. His-tagged E2-Crimson was synthesized in the microchambers array from digitalized DNA molecules and bound to NTA coated glass. After peeling off the PDMS chip, fluorescence signals for the arrayed spots were recorded. For the analysis, the averaged fluorescence intensity, which was recorded for each area where a PDMS microchamber was present, was taken into account. White dotted circles represent PDMS microchambers.

Supplementary references:

1. K. A. Underwood, J. R. Swartz and J. D. Puglisi, *Biotechnol Bioeng*, 2005, **91**, 425-435.
2. V. Kolb, E. Makeyev, W. Ward and A. Spirin, *Biotechnol Lett*, 1996, **18**, 1447-1452.