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Supplementary Materials for

Autonomous synthesis and assembly of a ribosomal subunit on a chip

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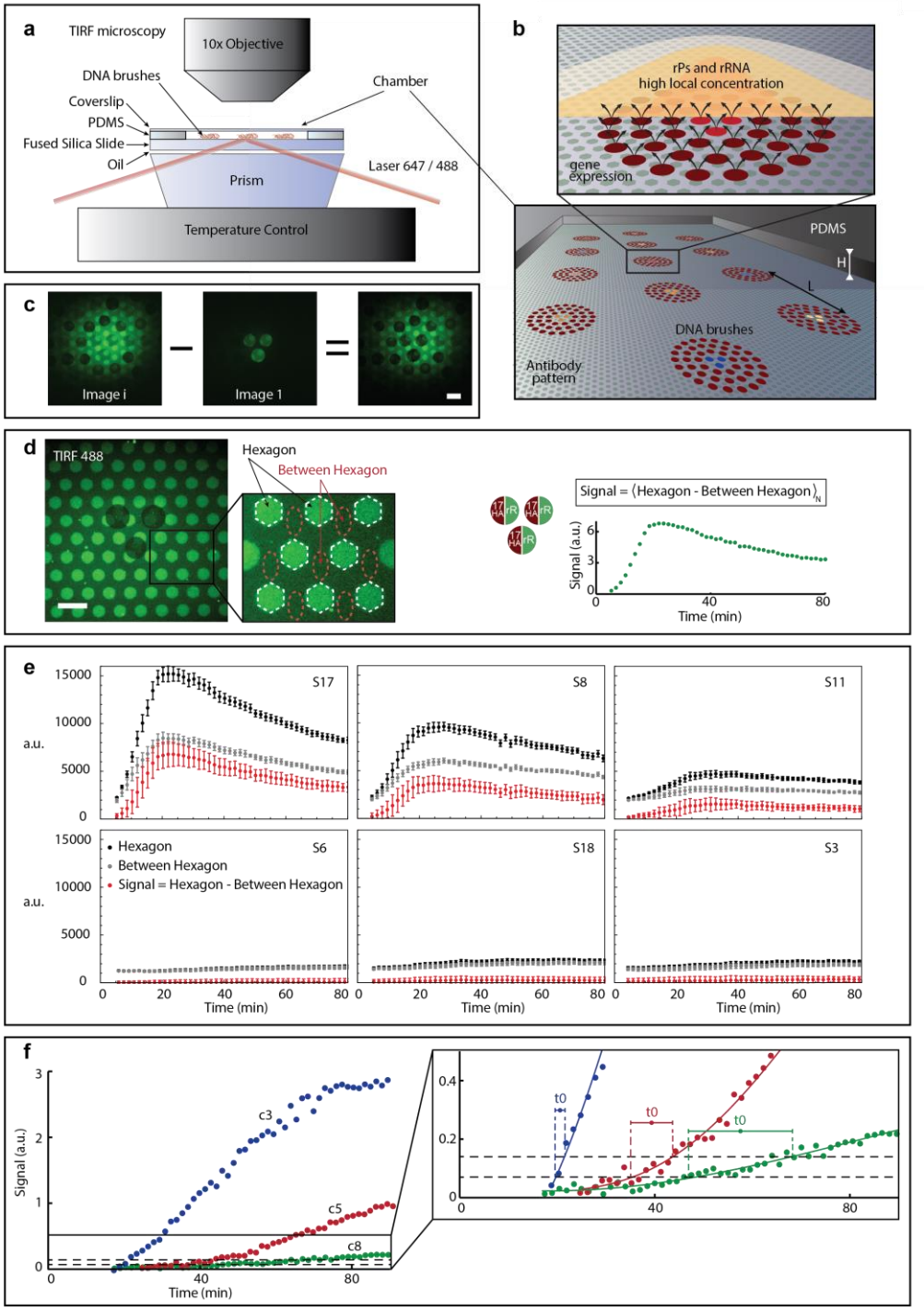

Fig. S1 Experimental setup and data analysis. (**a**) Scheme of experimental setup for TIRF imaging of the chip. (**b**) Bottom scheme, DNA brush configurations with a variable distance L organized in a chamber of variable height H. L and H are defined in Table S2 for each experiment. Top scheme, each brush is a local source of r-proteins and/or r-RNA. The proximity between brushes for a specific configuration gives rise to high local concentrations of products, favoring multi-molecular associations. (**c**) Background subtraction. At short times, r-RNA signal emerges only at the brushes containing the r-RNA gene, serving as a localized source for r-RNA transcription. Therefore, the first recorded image was subtracted from the rest of the images, resulting with only the r-RNA signal localized on surface antibodies. (**d**) Measurement of the dynamic fluorescent signal. At each time point, the fluorescent signal was the average of 3 to 10 different hexagons close to the three central brushes. The signal between hexagons was subtracted. (**e**) Examples of time dependent signals measured on the hexagons of the antibody pattern (black), between hexagons (red) and the differences between these two signals which correspond to the traces discussed in Fig. 1e. Error bars represent the standard deviation over 5-10 hexagons. (**f**) Evaluation of t_0, the signal starting time. The first data points were fitted using a polynomial function. The intersections between this function and two fixed thresholds define the interval containing t_0. Scale bar: 100µm

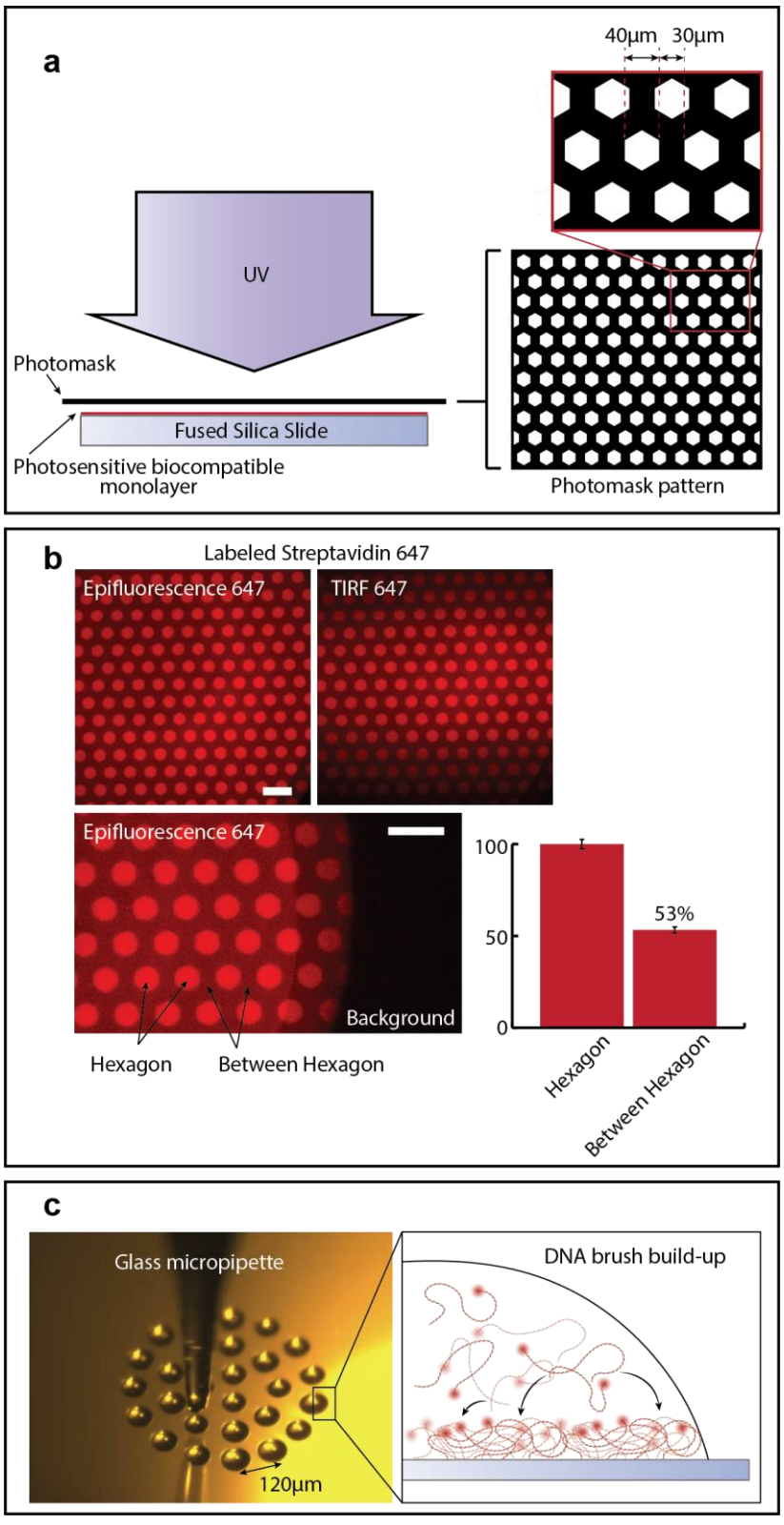

Fig. S2 Surface-patterning by UV Lithography and DNA deposition. (**a**) Scheme of the lithography process and details of the photomask. (**b**) Labeled streptavidin (647nm) on patterned biotin imaged in epifluorescence and TIRF microscopy. The laser beam illuminates homogeneously a region of 300x300µm. The streptavidin signal between hexagons reaches 53% of the signal inside hexagons, reflecting the relative level of activation of the photosensitive monolayer. (**c**) Nano-liter droplets containing SA-DNA conjugates are automatically deposited on the biotin-patterned surface with a 60µm glass micropipette. The DNA brushes are formed inside the droplets during incubation time. Scale bar: 100µm.

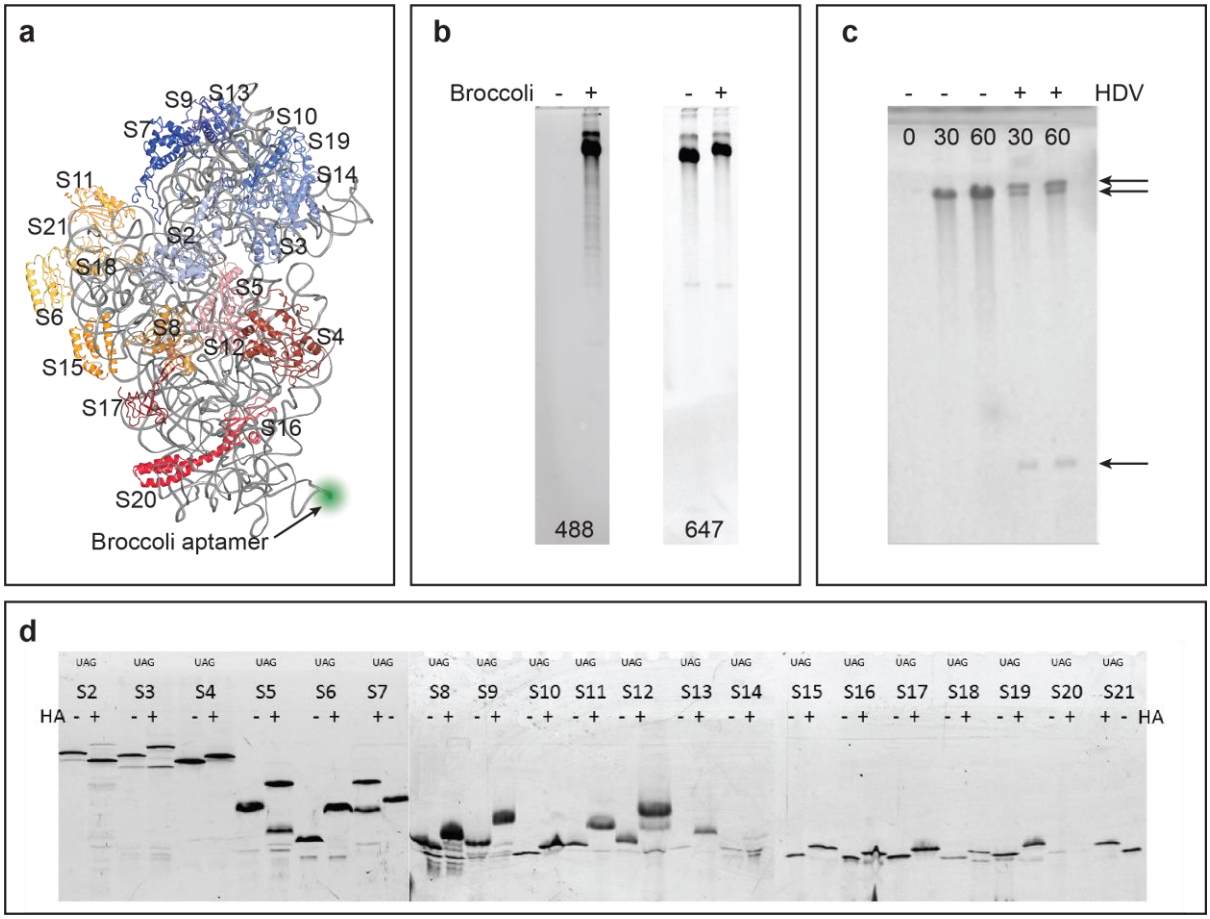

Fig. S3 r-RNA modifications and Cell-free synthesis of SSU r-proteins. (**a**) A ribbon diagram (PyMol Edu) of 16S r-RNA displaying the position of the r-proteins and position of Broccoli aptamer insertion into Helix6. Proteins are colored according to the general convention in this work. (**b**) r-RNA, with and without a Broccoli aptamer, were synthesized *in vitro* and *in situ* labeled with a 647-rUTP (647 image). Only the r-RNA -Broccoli band could be visualized by the Broccoli specific DHFBI dye (488 image) (Materials and Methods). (**c**) r-RNA with and without an HDV ribozyme were in vitro synthesized and resolved by gel electrophoresis after 30 and 60 mins. The position of the RNA fragments resulting from self-cleavage of the HDV ribozyme are marked by arrows. The efficiency of cleavage was approximately 50%. (**d**) All r-proteins-UAG (S2-S21) were expressed, each in a separate cell-free reaction, supplemented with a plasmid coding for non-tagged or HA-tagged r-protein. r-proteins were *in situ* labeled by the unnatural amino acid for simple detection in the gel (Methods).

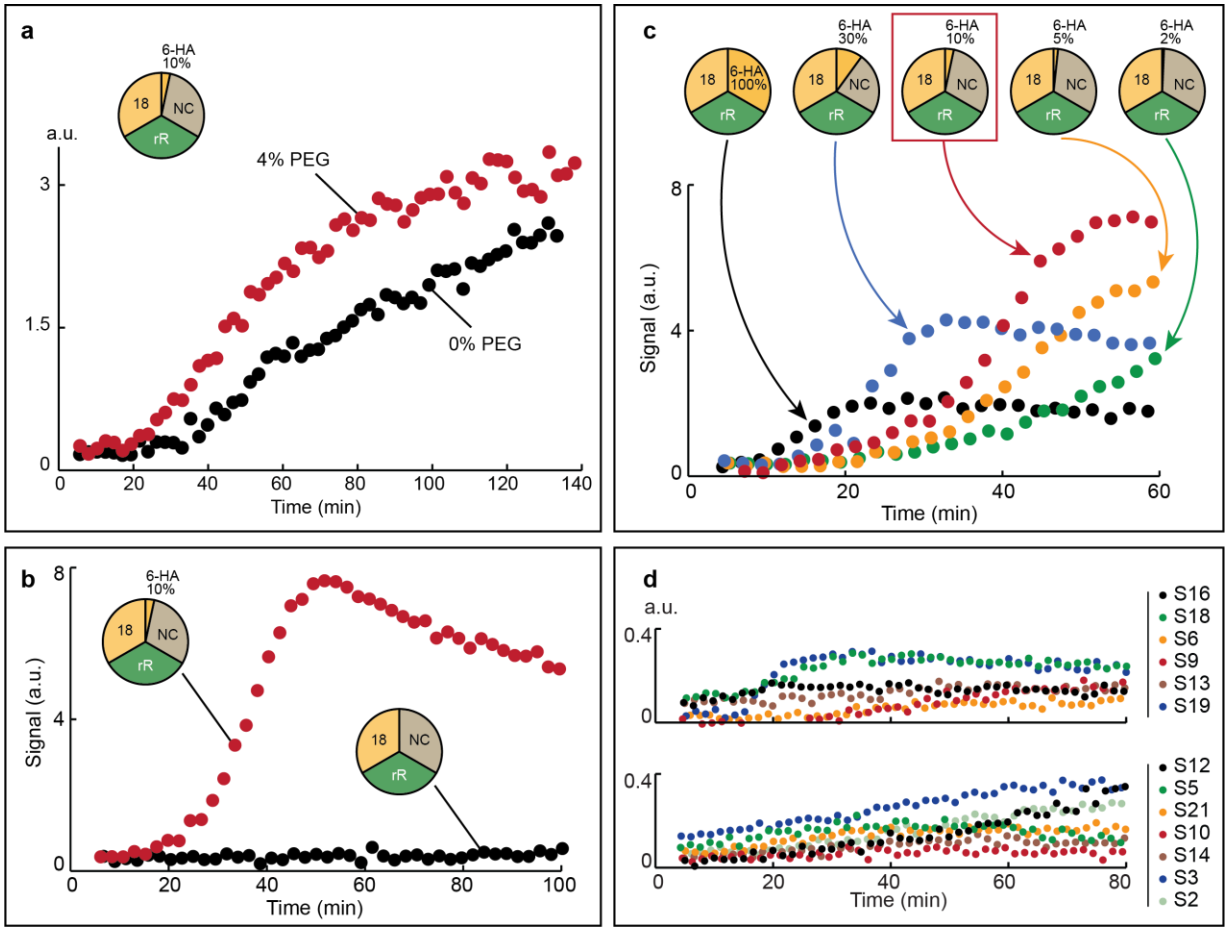

Fig. S4 Effect of crowding and gene ratio on r-RNA:S6-HA:S18 interaction. (**a**) Addition of PEG to on-chip expression reaction of r-RNA:S6-HA:S18 enhances the rate of signal appearance and overall intensity. (**b**) The r-RNA signal on surface antibodies with and without r-protein-HA genes. No non-specific signal was observed without r-protein-HA. (**c**) The ratio between S6-HA to r-RNA and S18 affected the rate of signal build-up on surface antibodies and overall intensity. A ratio of 1:10 S6-HA:r-RNA was found to have the highest signal intensity suggesting that the maximal number of r-RNA could be captured albeit with delayed kinetics. Excess r-protein-HA probably saturated antibodies, preventing those r-RNA that bound r-proteins-HA in the solution from binding to the saturated surface. The lag observed is most likely due to the lower amount of synthesized r-protein-HA that slows down the binding to the surface. Non-coding DNA (NC) was introduced to dilute the r-protein-HA gene. (**d**) A zoom of the two top right panels of Fig. 1e.

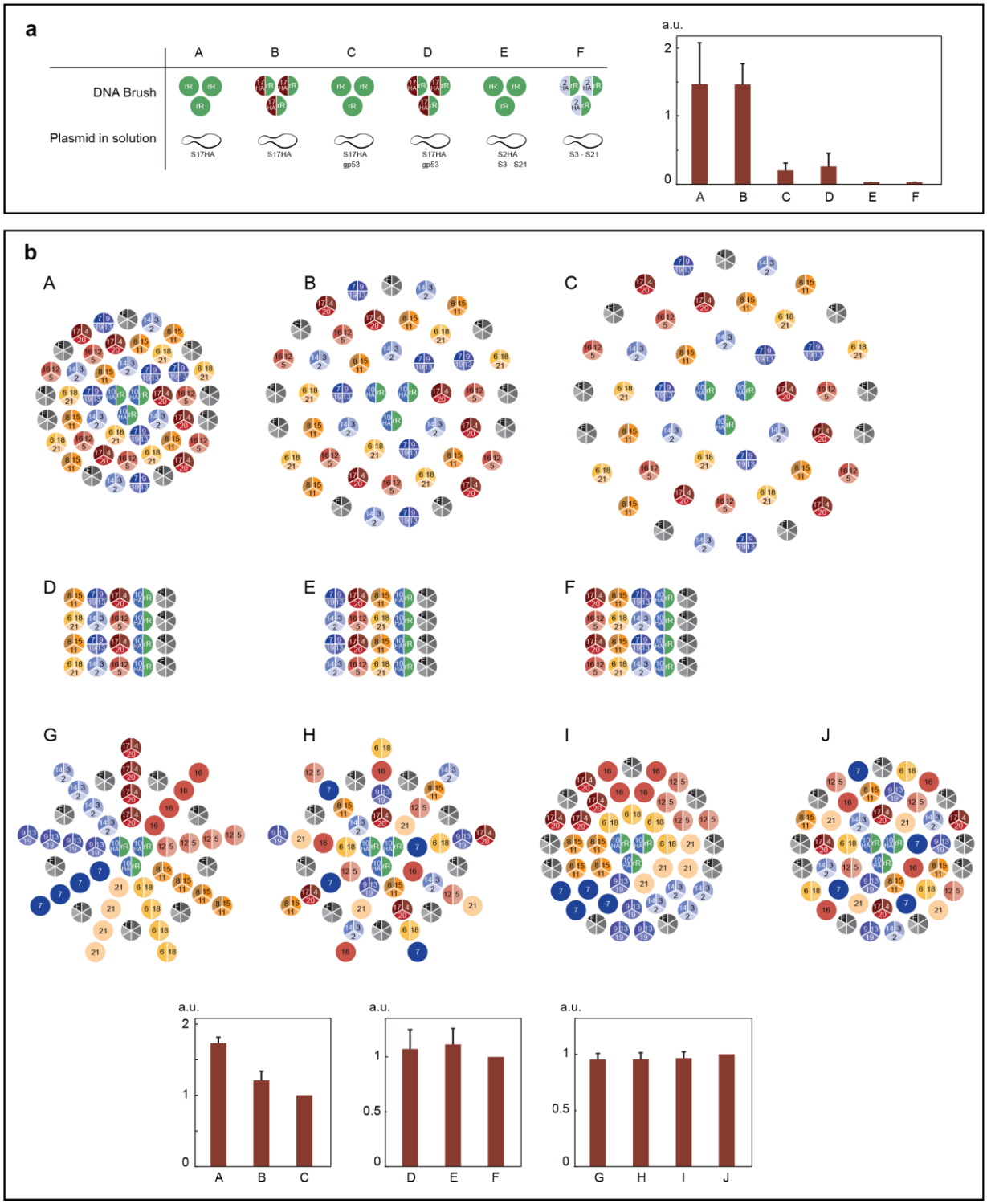

Fig. S5 Expression of r-proteins from plasmids in solution above the surface and effect of brush spatial organization. (**a**) Scheme: each experiment A-F with a different brush cluster: (A, C, E) r-RNA, (B, D) r-RNA:S17-HA, (F) r-RNA:S2-HA; and different plasmids in the solution: (A, B) S17-HA, 2nM (C, D) S17-HA + bacteriophage T4 gp53, 2nM and 38nM, respectively (E) S2-HA + S3-S21, each at 2nM, total of 40nM (F) S3-S21, each at 2nM. Right: Histograms f_{max} values of A-F. Error bars are standard deviations of 12 repeats for A, C, E and F (2 chips with 6 repeats each), and 4 repeats for B and D (2 chips with 2 repeats each). (**b**) Different spatial arrangements of *E. coli* r-protein genes, with S10-HA and r-RNA in all central brushes. Configurations B and C occupy a surface twice and three times larger than A, respectively, thereby changing DNA surface density. Configurations D, E and F permute the position of genes from the 5' (red), central (yellow) and 3' (blue) domains relative to the S10-HA:rRNA brushes. Assembly factors (gray-scale) remain on the right of the configurations. In A-F, genes are clustered as in Figure 1. In G- J, genes are clustered in the following way: 5' primary, 5' secondary, 5' tertiary, central primary, central secondary, central tertiary, 3' primary, 3' secondary, 3' tertiary, 6 assembly factors, S10-HA:r-RNA. The number of brushes was constant but they are reshuffled. In G and I, similar brushes are grouped together while in H and J, brushes are randomly organized. Histograms are of averaged f_{max} values of each layout. Error bars are standard deviation of three repeats.

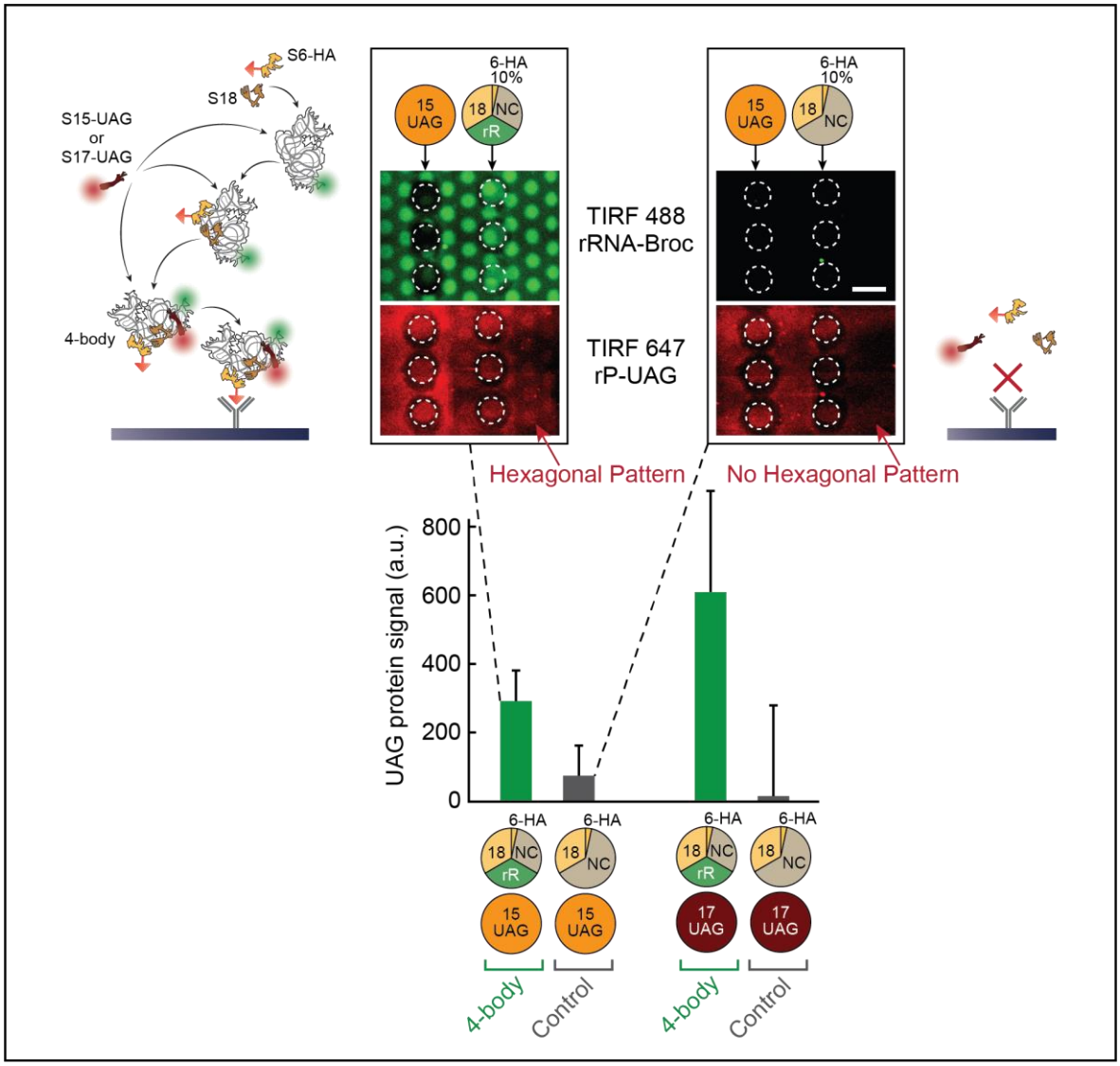

Fig. S6 Direct co-localization of r-RNA and r-proteins using fluorescent amino acid labeling. A line of brushes composed of genes coding for the primary r-protein UAG-S15 or UAG-S17 (coding for the incorporation of an unnatural fluorescent amino acid) was positioned next to a line of brushes coding either for the three components S6-HA, S18 and r-RNA, or for only S6-HA and S18. r-RNA Broccoli signal (TIRF excitation at 488nm) was observed next to the 3-body brushes, indicating the 3-body assembly on the patterned antibodies (scheme). No pattern was observed in absence of r-RNA (top right picture). A r-protein-UAG signal (TIRF excitation at 647nm) was observed only in presence of the r-RNA, demonstrating the direct binding of the primary r-protein-UAG as a 4-body complex (bottom left and right pictures). Fluorescent signals of labeled r-proteins were recorded at t=135min and t=85min for S15-UAG and S17-UAG, respectively, with (green bars) and without (grey bars) r-RNA. NC: non-coding DNA. Scale bar: 100µm.

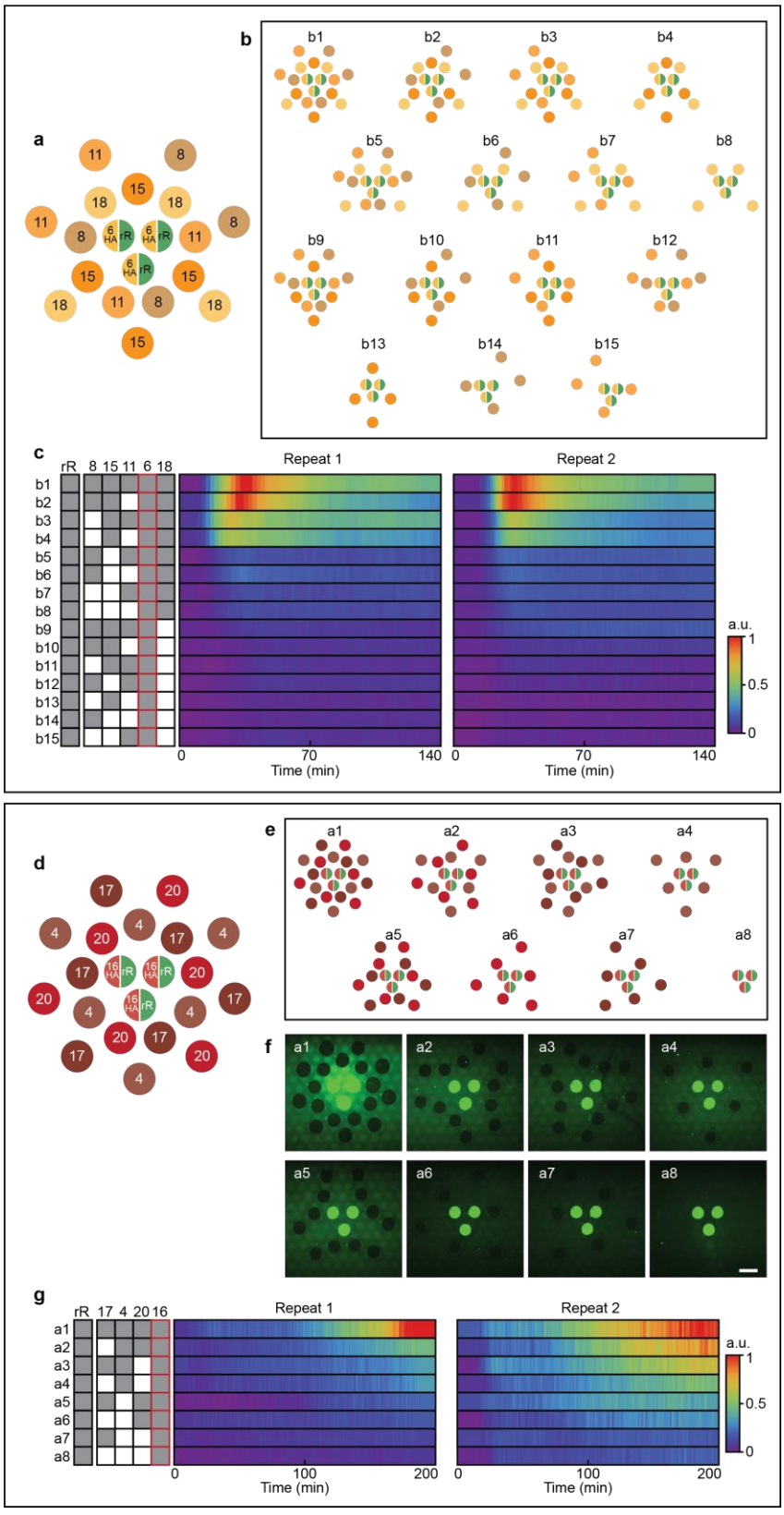

Fig. S7 Interactions of r-RNA with S6-HA and S16-HA with different configurations of central and 5' domain r-proteins, respectively. (**a**, **b**, **c**) r-RNA:S6-HA interaction. (**a**) Spatial arrangement of the DNA brushes when all the six genes involved are present. (**b**) The different configurations of the study. (**c**) Repeats of the experiment presented Fig. 3c. (**d**, **e**, **f**, **g**) rRNA:S16-HA interaction. (**d**) Spatial arrangement of the DNA brushes when all the five genes involved are present. (**e**) The different configurations of the study. (**f**) TIRF images of the configurations at t=150min. Scale bar: 100μm. (**g**) Repeats of the experiment presented Fig. 3d.

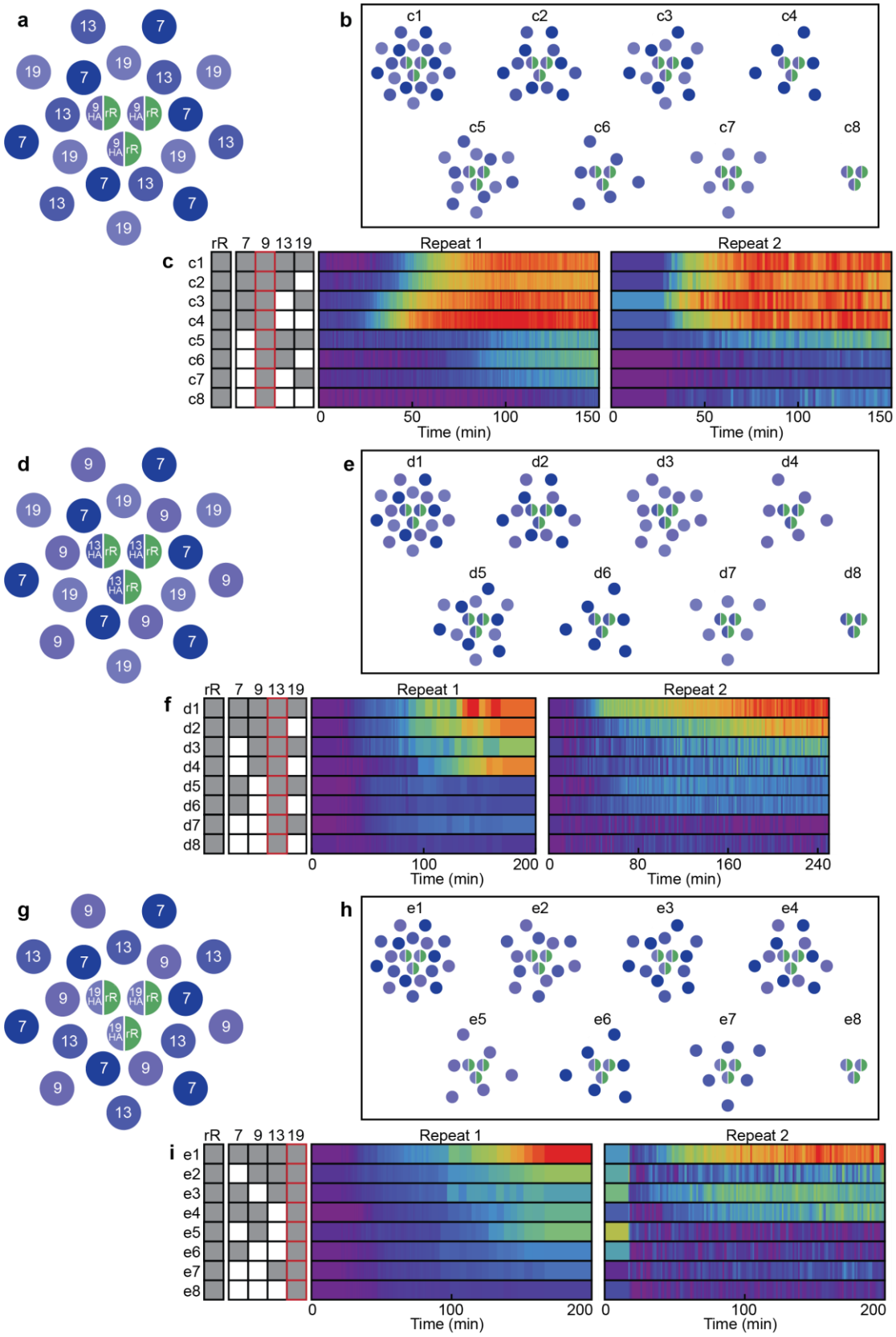

Fig. S8 r-RNA interactions with S9-HA, S13-HA and S19-HA. (**a**, **d**, **g**) Spatial arrangement of the DNA brushes when all the five genes involved are present. (**b**, **e**, **h**) The different configurations of the studies. (**c**, **f**, **i**) Repeats of the experiments presented Fig. 3e.

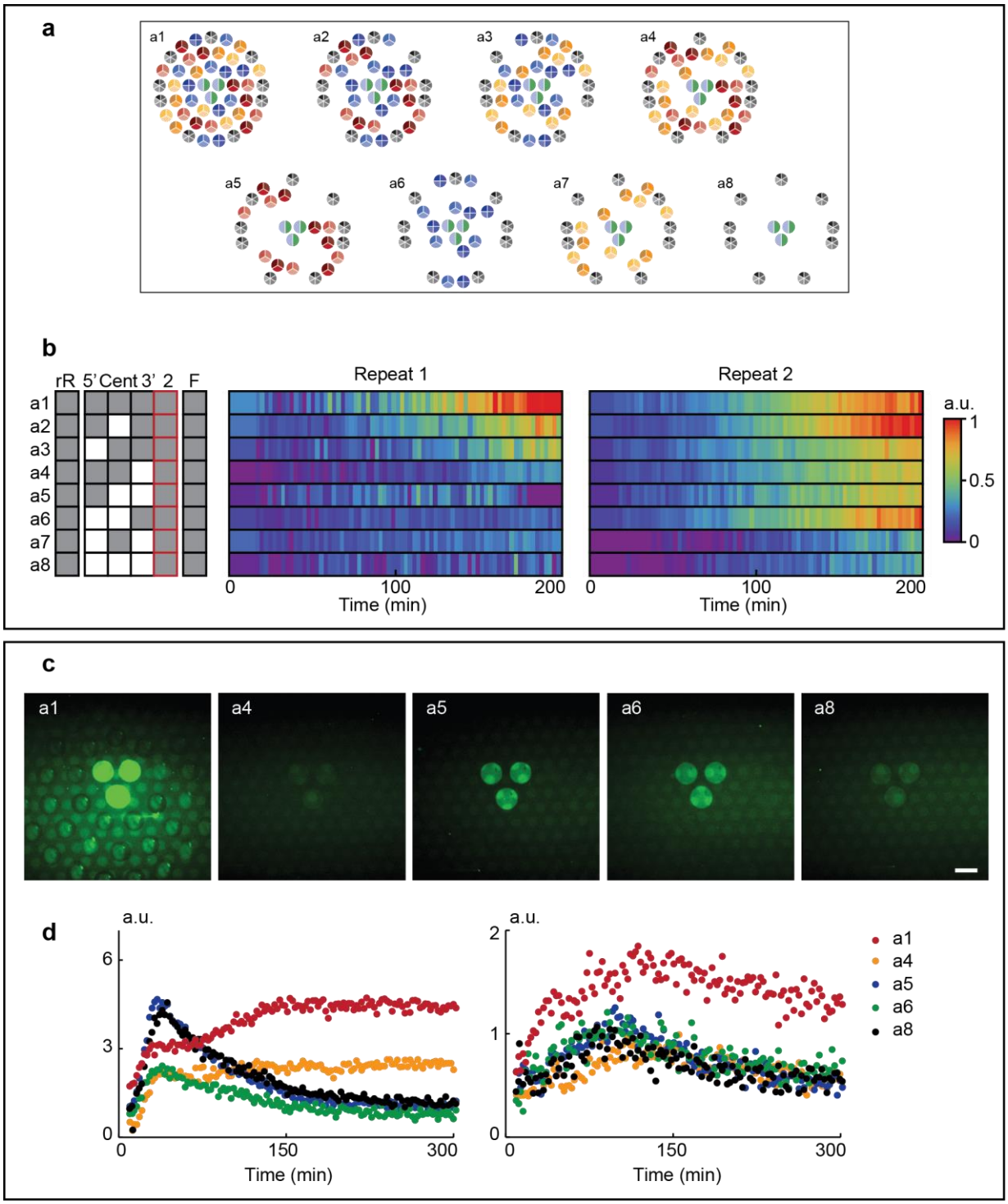

Fig. S9 r-RNA:S2-HA interactions with 6 and 4 factors, and different domain deletions. (**a**) The different configurations of the study with 6 factors. (**b**) Repeats of the experiment presented Fig. 4a. (**c**) Study with 4 factors. TIRF images of the configurations at t=350min. Scale bar: 100µm. (**d**) Repeats of the experiment presented Fig. 4c.

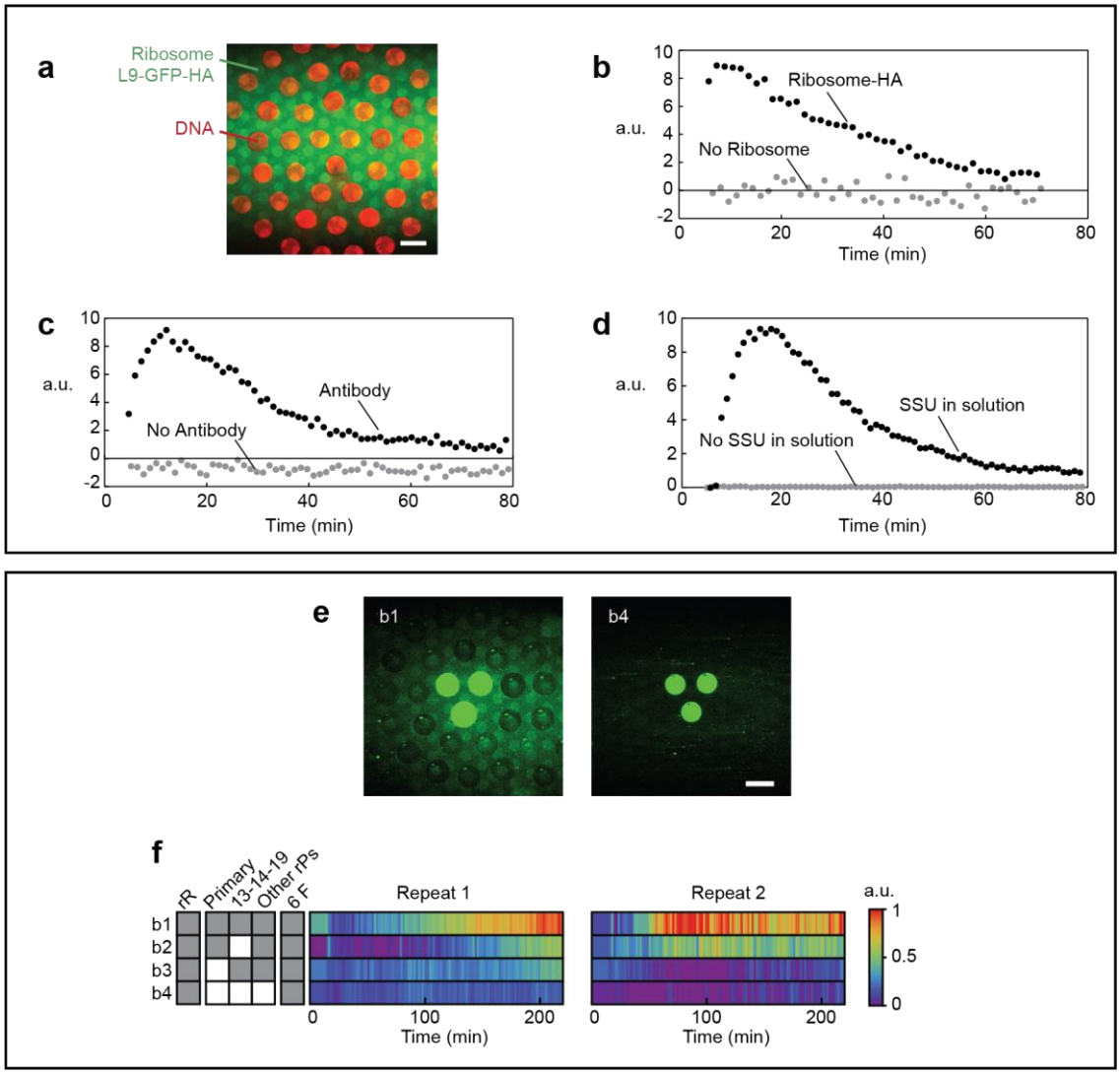

Fig. S10 Interaction of nascent SSU with surface-immobilized LSU. (**a**) Fluorescent image presenting the arrangement of the DNA brushes (red, epifluorescence excitation 647nm) and surface immobilized ribosomes (green, TIRF excitation 488nm) for the experiment described in Figs. 4e,f. Ribosomes modified with a L9-GFP-HA protein were used here when ribosomes modified with a L9- HA protein (no GFP) were used in the experiment described in Fig. 4d-f. Scale bar: 100µm. (**b**, **c**, **d**) Fluorescent signals on surface immobilized ribosomes localized next to DNA brushes coding for GFP-SecM (black circles, see Fig. 4d) compared to the following negative controls (gray circles): (**b**) the ribosomes are not modified with an HA tag (No Ribosome), (**c**) the antibodies are not added to the chamber (No Antibody) and (**d**) the reaction was not supplemented with purified SSU (No SSU in solution). (**e**) TIRF images of the configurations b1 and b4 at t=220min. Scale bar: 100µm. (**f**) Repeats of the experiment presented Fig. 4f.

Table S1 Primers used for cloning r-proteins and r-RNA *E. coli* genes.

Name	Sequence
F16S rRNA	GCGAAATTAATACGACTCACTATAGGGTAAATTGAAGAGTTTGATCATGGCTC
R16S rRNA	AAAGGCCTCCTGCAGGTTAACCTTACTCGAGTAAGGAGGTGATCCAACCGCAG
F16S HDV	TGGCGGCTAGTGGGCAACATGCTTCGGCATGGCGAATGGGACGTAACTAGCATAACCCCTTGGGGCC
R16S HDV	GCGAGGAGGCTGGGACCATGGCTAGCTAAGGAGGTGATCCAACCGCAGGTTCCCCTAC
F16S Broccoli	GTATCTGTCGAGTAGAGTGTGGGCTCCGCTGCTTCTTTGCTGACGAGTGGCGGAC
R16S Broccoli	GAATATCTGGACCCGACCGTCTCCGCAGCTTCTTCCTGTTACCGTTCGAC
FS2	F-Cons.seq-ATGGCAACTGTTTCCATGCGCG
RS2	R-Cons.seq-CTCAGCTTCTACGAAGCTTTC
FS3	F-Cons.seq-ATGGGTCAGAAAGTACATCCTAATG
RS3	R-Cons.seq-TTTACGGCCTTTACGCTGC
FS4	F-Cons.seq-ATGGCAAGATATTTGGGTCCTAAG
RS4	R-Cons.seq-CTTGGAGTAAAGCTCGACGATC
FS5	F-Cons.seq-ATGGCTCACATCGAAAAACAAGC
RS5	R-Cons.seq-TTTCCCCAGAATTTCTTCAACGG
FS6	F-Cons.seq-ATGCGTCATTACGAAATCGTTTTTATGG
RS6	R-Cons.seq-CTCTTCAGAATCCCCAGCATC
FS7	F-Cons.seq-ATGCCACGTCGTCGCGTCATT
RS7	R-Cons.seq-CCAACGGTAGTGTGCGAACG
FS8	F-Cons.seq-ATGAGCATGCAAGATCCGATCGC
RS8	R-Cons.seq-GGCTACGTAGCAGATAATTTCGCC
FS9	F-Cons.seq-ATGGCTGAAAATCAATACTACGGC
RS9	R-Cons.seq-ACGTTTGGAGAACTGCGGACG
FS10	F-Cons.seq-ATGCAGAACCAAAGAATCCGTATCC
RS10	R-Cons.seq-ACCCAGGCTGATCTGCACGTC
FS11	F-Cons.seq-ATGGCAAAGGCACCAATTCGTGC
RS11	R-Cons.seq-TACGCGACGTTTTTTCGGCGG
FS12	F-Cons.seq-ATGGCAACAGTTAACCAGCTGGTAC
RS12	R-Cons.seq-AGCCTTAGGACGCTTCACGCC
FS13	F-Cons.seq-ATGGCCCGTATAGCAGGCATTAAC
RS13	R-Cons.seq-TTTCTTGATCGGTTTGCGCGG
FS14	F-Cons.seq-ATGGCTAAGCAATCAATGAAAGC
RS14	R-Cons.seq-CCAGCTAGCCTTTTTCAGACCC
FS15	F-Cons.seq-ATGTCTCTAAGTACTGAAGCAACAG
RS15	R-Cons.seq-GCGACGCAGACCCAGG
FS16	F-Cons.seq-ATGGTAACTATTCGTTTAGCACGTC

RS16	R-Cons.seq-AGCTGCTTTGTTTACTTCTTTGATC
FS17	F-Cons.seq-ATGACCGATAAAATCCGTACTCTGC
RS17	R-Cons.seq-CAGAACCGCTTTCTCTACAACG
FS18	F-Cons.seq-ATGGCACGTTATTTCCGTCGTCGC
RS18	R-Cons.seq-CTGATGGCGATCAGTGTACGGC
FS19	F-Cons.seq-ATGCCACGTTCTCTCAAGAAAGG
RS19	R-Cons.seq-TTTCTTCTTCGCTTTTTTATCAGCAG
FS20	F-Cons.seq-ATGGCTAATATCAAATCAGCTAAGAAGCG
RS20	R-Cons.seq-AGCCAGTTTGTTGATCTGTGC
FS21	F-Cons.seq-ATGCCGGTAATTAAAGTACGTGAAAAC
RS21	R-Cons.seq-GTACAGACGAGTGCGGCGTG
F-Cons.seq-PURExpress control plasmid	GTTTAACTTTAAGAAGGAGATATACAT
R-Cons.seq-PURExpress control plasmid	AAAGGCCTCCTGCAGGTTAACCTTACTTA
F- Cons.seq pIVEX2.5	GTTTAACTTTAAGAAGGAGATATACATATGTAG
R- Cons.seq pIVEX2.5	GGGTAGCTGGTCCCGGGAGCTCGCTT
L9	CTTTAAGAAGGAGATATACATATGCAAGTTATTCTGCTTGAT
RL9	GCTGGTCCCGGGAGCTCGCTTTTCAGCTACTACGTTTACGATC

Table S2 Experimental parameters.

Experiment	Number of genes	Configuration diameter D (μm)	Center-to-center distance between nearest configurations (L_X, L_Y) (mm)	Chamber height H (μm)	Estimated time of diffusion between configurations* $t_{max}=L^2/4D_0$ (min)
Fig. 1	2	120	(2, 2)	100	330
Fig. 2	27	840	(2.5, 3)	50	520
Fig. 3	2 to 6	540	(2.5, 2.5)	100	520
Fig. 4a	8 to 27	840	(4, 4)	50	1330
Fig. 4b	2 to 27	840	(2.7, 6)	50	600
Fig. 4c	6 to 25	840	(4.3, 4.3)	50	1540
Fig. 4f	7 to 28	1080	(3.5, 3.5)	50	1020

*To avoid crosstalk between brush configurations in the same chamber due to diffusion of molecules, we calculated the time $t_{max} = L^2/4D_0$ for a typical r-Protein to diffuse a distance L separating two configurations (center-to-center), with $D_0 = 50\mu m^2/s$, an estimated diffusion coefficient of a typical r-protein. The experimental analysis was always performed for $t < t_{max}/2$.

Supplementary Video files

Movie S1 DNA deposition on the chip.

Spotting of nano-liter droplets of SA conjugated DNA solutions in an automatic way on the biotin-patterned chip surface using a 60µm diameter micropipettes. Each droplet is spotted three times to increase reproducibility between droplets. The movie is sped up four times.

Movie S2 r-RNA signal build-up: 2-body.

TIRF time-lapse images showing the r-RNA signal emerging and propagating from central brushes composed of r-RNA and S8-HA genes (a primary r-protein) in a 10:1 ratio in the absence of any other gene (Fig. 1c,d). The first image was subtracted from all images as discussed in Fig. S1. Brush diameter 95µm. Film duration 80min.

Movie S3 2-body signal build-up for all r-protein-HAs organized according to the established SSU assembly map.

TIRF time-lapse images showing the r-RNA signal emerging and propagating for all r-RNA:r-protein-HA configurations discussed in Fig. 1c,d, in the absence of other r-proteins. The images are arranged according to the assembly map, which emphasizes the distinction between primary r-proteins presenting strong signals and secondary and tertiary r-proteins exhibiting much weaker signals. The first image was subtracted from all images as discussed in Fig. S1. Brush diameter 80µm. Film duration 70min.

Movie S4 r-RNA signal build-up: N-body.

TIRF time-lapse images showing the r-RNA signal emerging and propagating from central brushes composed of r-RNA and S15-HA genes in a 10:1 ratio in presence of all other r-proteins and assembly factors expressed from radially organized brushes as described in Fig. 1. The first image was subtracted from all images as discussed in Fig. S1. Brush diameter 85µm. Film duration 80min.

Movie S5 r-RNA signal build-up for all r-protein-HAs organized according to the established SSU assembly map.

TIRF time-lapse images showing the r-RNA signal emerging and propagating for every r-protein-HA in the presence of all other r-proteins (Fig. 2). The images are arranged according to the assembly map which emphasize the order of assembly. The first image was subtracted from all images as discussed in Fig. S1. Brush diameter 85µm. Film duration 140min.

Movie S6 Effect of r-proteins on r-RNA:S2-HA interaction in the presence of the 2 GTPase assembly factors.

(Left panel) TIRF time-lapse images showing the r-RNA signal emerging and propagating from central brushes composed of r-RNA and S2-HA genes in a 10:1 ratio in the presence of the 2 GTPase factors. (Right panel) The same configuration as on left panel in the presence of all other r-proteins, organized as described in Fig. 1b. The r-proteins enhanced significantly the signal. The first image was subtracted from all images as discussed in Fig. S1. Brush diameter 85µm. Film duration 220min.

Movie S7 Stable and unstable r-RNA:S2-HA interactions.

(Right panel) TIRF time-lapse images showing the r-RNA signal emerging and propagating from central brushes composed of r-RNA and S2-HA genes in a 10:1 ratio in the presence of 4 assembly factors with no GTPase

activity. A signal promptly built up but was unstable and disappeared in time. (Left panel) the same configuration as in right panel with addition of all the other r-proteins, organized as in Fig. 1b. A slower but stable r-RNA signal appeared, as discussed in Fig. 4c. The first image was subtracted from all images as discussed in Fig. S1. Brush diameter 90μm. Film duration 400min.