Electronic Supplementary Material (ESI) for Molecular BioSystems

Electronic Supporting Information

A controllable gene expression system in liposomes that includes a positive feedback loop

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Materials and Methods

DNA fragments

The DNA fragment encoding GFP and inactive T3 RNA polymerase for a control experiment were prepared by PCR using primers, atcggtgatgtcggcgatatag and gctagttattgctcagcgg, and the plasmid pETg5-T3 and pETT3-T3mut, respectively, as a template. The DNA fragments encoding active T3 RNA polymerase was prepared by PCR using primers, aattaaccctcactaaaggggaattgtgagcgg and ctcctttcagcaaaaaacccctcaagaccc, and the plasmid pETT3-T3 as a template. The plasmid pETg5-T3 was constructed by changing the T7 promoter in pETG5tag[2] into T3 promoter. The plasmid pETT3-T3 was constructed by changing GFP gene into T3 RNA polymerase gene. The plasmid pETT3-T3mut was constructed by inserting the point mutantion of T3 RNA polymerase (C2442A, S814R) into pETT3-T3.

DNA fragments

The DNA fragment encoding GFP was prepared by PCR using the primers atcggtgatgtcggcgatatag and ccgctgagcaataactagc and the plasmid pETg5-T3 as a template. The DNA fragments encoding T3 RNA polymerase and an inactive RNA polymerase as a control were prepared by PCR using the plasmids pETT3-T3 and pETT2-T3mut as the templates, respectively, and the primers aattaaccctcactaaaggggaattgtgagcgg and ctcctttcagcaaaaaacccctcaagaccc. The plasmid pETg5-T3 was constructed by replacing the T7 promoter in pETG5tag[2] with a T3 promoter. The plasmid pETT3-T3 was constructed by replacing the GFP gene with the T3 RNA polymerase gene. The plasmid pETT2-T3mut was constructed by inserting point mutations into the T3 RNA polymerase sequence (C2442A and S814R) in pETT3-T3.

Translation system

The reconstituted translation system was prepared by mixing each protein component at the concentration reported previously[3], except that T3 RNA polymerase (0.055 units/μl, Promega) was used instead of T7 RNA polymerase. The composition of the low-molecular-weight compounds was changed as follows: 18 amino acids (0.3 mM), tyrosine (0.3 mM), cysteine (0.3 mM), tRNA mix (2.9 μg/μl), ATP (2 mM), GTP (2 mM), CTP (1 mM), UTP (1 mM), HEPES-KOH (pH 7.6, 100 mM), potassium glutamate (280 mM), spermidine (2 mM), magnesium acetate (8.55 mM), creatine

phosphate (20 mM), dithiothreitol (1 mM), and 10-formyl-5,6,7,8-tetrahydrofolic acid (10 ng/μl). The purified LacI protein was prepared from recombinant *Escherichia coli* harboring a plasmid encoding LacI using the method that was used for the HrpA protein in a previous study[4]. IPTG was purchased from Wako (Japan).

Switching reaction under bulk conditions

We mixed each DNA fragment (3 nM) with the translation system containing purified LacI (1 μM). For the experiment without the PFL, we added the DNA fragment encoding the inactive mutant T3 RNA polymerase instead of the fragment with the intact gene. This mutant T3 RNA polymerase contains two point mutations (C2442A and S814R) and lacks transcriptional activity (Fig. S2). The incubation of the mixture at 37°C and the measurement of GFP fluorescence were performed simultaneously using the Mx3005P qPCR system (Agilent Technologies). The fluorescence was measured every minute.

Switching reaction in liposomes

We mixed each DNA fragment (3 nM) with the translation system containing purified LacI (148 nM) and Alexa 647-labeled transferrin (2.8 μM, Invitrogen) as a volume indicator. The mixture was encapsulated in liposomes (POPC:cholesterol = 9:1) using a previously reported method[1] modified by the addition of α-hemolysin (5 μM, Sigma) after liposome preparation. The liposomes were incubated with IPTG at 37°C, and the levels of green and red fluorescence were measured using a flow cytometer (FACSAria, BD). Based on the red fluorescence, the liposome volumes were estimated as described previously[1].

Kinetics of GFP fluorescence with the PFL

In the switching reaction without PFL, the concentrations of each component were calculated as follows:

d[RNA_gfp]/dt =k_1 [Protein_t3rnap],

d[Protein_gfp]/dt = $k2$ [RNA_gfp],

where [RNA_gfp], [Protein_gfp], and [Protein_t3rnap] represents the concentrations of the RNA encoding GFP, the GFP protein, and the T3 RNA polymerase protein, respectively. k_1 and k_2 represent the transcription and translation rate constants of the gfp gene, respectively. The DNA concentration is included in the rate constant k_1.

Because [Protein_t3rnap] is constant over time in this case, these equations are solved as

$$[\mathrm{Protein\_gfp}] = \frac{k_1 k_2 [\mathrm{Protein\_t3rnap}]_{t=0}}{2} t^2 .$$

The second derivative of this equation is

$$\frac{d^2[\text{Protein\_gfp}]}{dt^2} = k_1 k_2 [\text{Protein\_t3rnap}]_{t=0},$$

which is also constant over time.

In the case of the switching reaction with the PFL, the concentrations of each component were calculated as follows:

d[RNA_gfp]/dt =k_1 [Protein_t3rnap],

d[Protein_gfp]/dt = k_2 [RNA_gfp],

d[RNA_t3rnap]/dt =k_3 [Protein_t3rnap],

d[Protein_t3rnap]/dt =k_4 [RNA_t3rnap],

where [RNA_t3rnap] represents the concentration of the RNA encoding T3 RNA polymerase, and k_3 and k_4 represent the transcription and translation rate constants for the T3 RNA polymerase gene, respectively.

These equations are solved as:

$$[\text{Protein\_gfp}] = \frac{k_1 k_2 [\text{Protein\_t3rnap}]_{t=0}}{2k_3 k_4} e^{-\sqrt{k_3 k_4}t} (-1 + e^{\sqrt{k_3 k_4}t})^2 .$$

The second derivative of this equation is given as:

$$\frac{d^2[\text{Protein\_gfp}]}{dt^2} = \frac{k_1 k_2 [\text{Protein\_t3rnap}]_{t=0}}{2} (e^{-\sqrt{k_3 k_4}t} + e^{\sqrt{k_3 k_4}t}).$$

The expansion of the exponential functions gives

$$\frac{d^2[\text{Protein\_gfp}]}{dt^2} = \frac{k_1 k_2 [\text{Protein\_t3rnap}]_{t=0}}{2} (2 + k_3 k_4 t^2 + \frac{k_3^2 k_4^2 t^4}{12} + \cdots),$$

which is a function that increases over time with a concave curve.

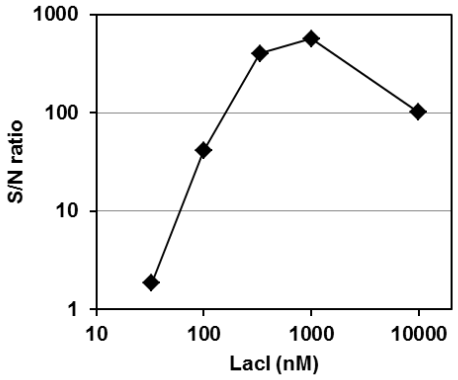

Fig. S1 The effect of the LacI concentration on the S/N ratio. The switching experiment with the

PFL was performed with various concentrations of LacI. The ratio of the GFP fluorescence after 180 min of incubation with IPTG (900 μM) to the GFP fluorescence without IPTG was plotted as the S/N ratio.

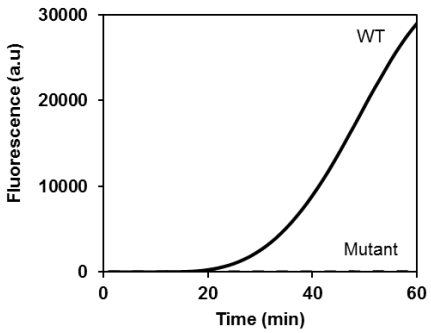

Fig. S2 The transcriptional activity of the mutant T3 RNA polymerase. The RNA fragments encoding the wild-type T3 RNA polymerase gene or the mutant T3 gene (C2442A and S814R) were mixed with the translation system containing the DNA fragment encoding the gfp gene under the control of the T3 promoter. After incubation, the GFP fluorescence was measured every minute.

References

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