# Supplementary Files

## Regulation of spatiotemporal patterning in artificial cells by a defined protein expression system

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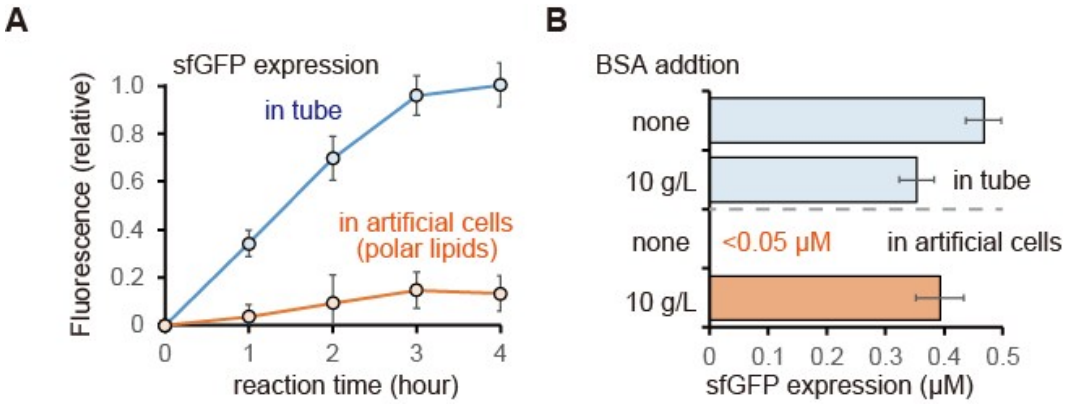
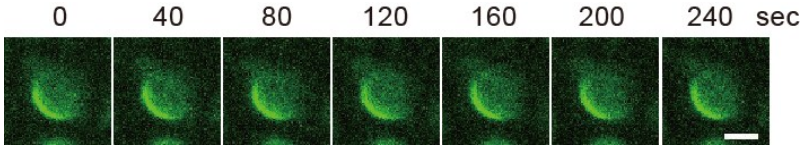
Contents:

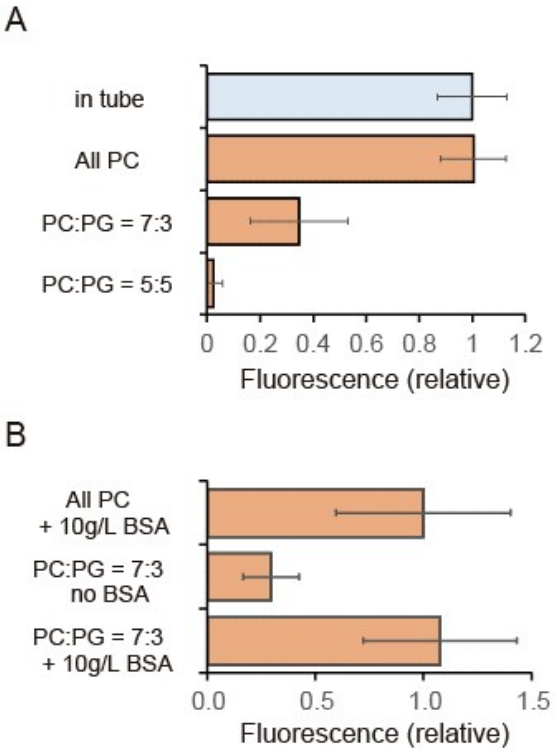



**Supplementary Figure S1. Recovery of protein synthesis levels in artificial cells by supplementation of high concentration BSA.**

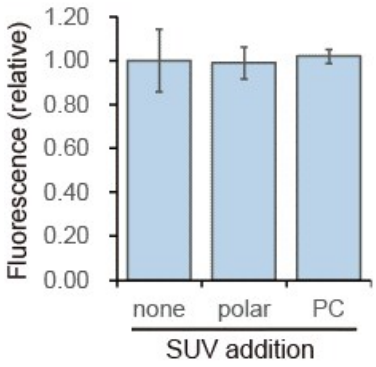
A: Time plots of relative fluorescent intensities of sfGFP synthesized by a normal PURE system in tubes or in artificial cells. Fluorescent intensities were normalized to the value of that in tube synthesis at 4 h. To compare the levels of sfGFP synthesized in tubes and artificial cells, solutions for tube reaction were encapsulated in artificial cells after in-tube synthesis. Error bars indicate standard deviation (N = 30). B: Restoring protein synthesis levels by addition of 10mg/mL BSA. The amounts of sfGFP synthesized were estimated from a standard curve obtained using purified sfGFP. Blue and orange bars indicate amounts sfGFP synthesis in tubes and in artificial cells after 4 h at 37°C, respectively. Error bars indicate standard deviation (N = 30).



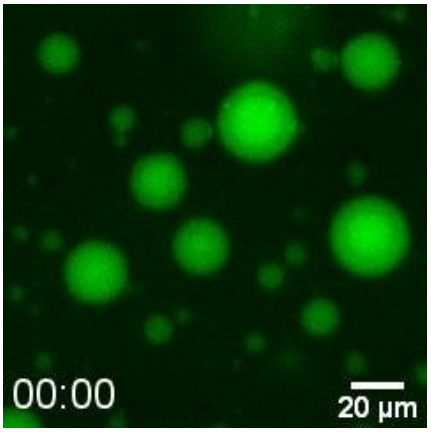

**Supplementary Figure S2. Time-lapse images of inhomogeneous membrane localization of msfGFP-MinC in artificial cells.** Scale bar indicates 20 μm.



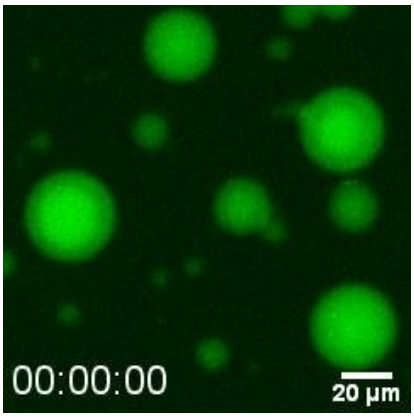

**Supplementary Figure S3.** Effects of negative lipids on protein expression levels in artificial cells. A: Fluorescence levels of sfGFP synthesized after 2h protein expression by the PURE system are shown. The levels were normalized by the average of in the case tube reaction. Error bars indicate standard deviation (n=30 droplets). B: Fluorescence levels of sfGFP synthesized after 4h protein expression in artificial cells by the PURE system with or without 10 g/L BSA are shown. The levels were normalized by the average level in All PC with 10g/L. A&B: All PC indicate protein synthesis in artificial cells covered with POPC. PC:PG indicate the ratio of the lipid mixtures for preparation of artificial cells. PG indicates DOPG. Error bars indicate standard deviation (n=30 droplets).



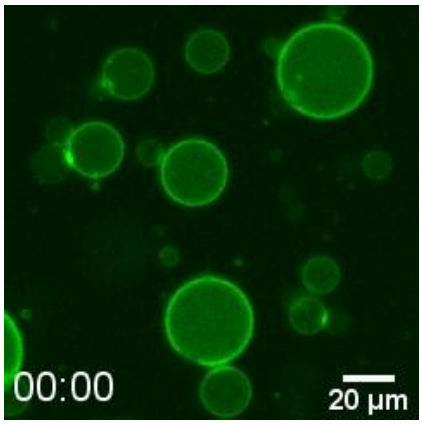

**Supplementary Figure S4.** Effects of external addition of lipids on protein expression levels in tubes. SUVs made of E. coli polar lipids (polar) or POPC (PC) are supplied in the reaction mixtures for the PURE system (in tube reaction). The fluorescent levels of sfGFP synthesized detected by non-boil SDS-PAGE were normalized by the average level in the case of no SUV addition. Error bars indicate standard deviation (n=3 tubes).



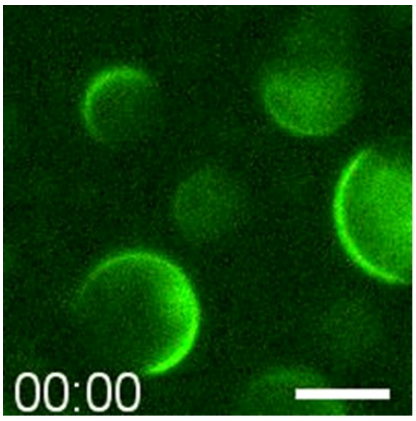

**Supplementary Movie S1.** Changes of spatiotemporal patterns by MinD synthesis in artificial cells (1 nM MinD DNA for protein synthesis)



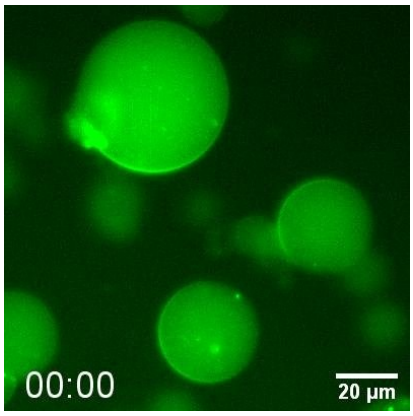

**Supplementary Movie S2.** Changes of spatiotemporal patterns by MinE synthesis in artificial cells (Purified 1 μM MinE protein and 5 nM MinD DNA for protein synthesis)




**Supplementary Movie S3.** Changes of spatiotemporal patterns by MinE synthesis in artificial cells (Purified 1 μM MinD protein and 1 nM MinE DNA for protein synthesis)




**Supplementary Movie S4.** Min waves generated by protein synthesis using the MinDE DNA operon. The Min wave in an artificial cell at the upper right is pole-to-pole oscillation, and others are traveling waves. Scale bar indicates 15 μm.




**Supplementary Movie S5.** Regulation of in Min waves generation in liposomes *via* protein synthesis induced by IPTG. Liposomes were made of the PC/PG lipid mixture described in Experimental. Scale bar: 15 μm.