Supporting Information

Gene Circuit Compartment on Nano-interface Facilitates Cascade Gene Expression

Xiaocui Guo[1]‡, Feng Li[1]‡, Lihui Bai[1], Wenting Yu[1], Xue Zhang[1], Yi Zhu[1], and Dayong Yang[1]*

[1]Frontier Science Center for Synthetic Biology, Key Laboratory of Systems Bioengineering (MOE), School of Chemical Engineering and Technology, Tianjin University, Tianjin, 300350, P.R. China.

E-mail: dayong.yang@tju.edu.cn

‡These authors contributed equally.

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EXPERIMENTAL SECTION

Materials

pQE-T7 RNAP plasmid and pRset5d-eGFP plasmid were preserved in our lab. Oligonucleotides were synthesized by GENEWIZ (Beijing, China), Hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot 4H_2O$) was from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China), Sodium citrate was purchased from Solarbio, L-ascorbic acid was purchased from Dingguo changsheng biotechnology (Beijing, China), 2×Pfu PCR MasterMix, TIANquick Midi Purification Kit (DP204) and DNA marker were purchased from TIANGEN (Beijing, China), 4',5',8'-trimethypsoralen was from ThermoFisher. *E.coli BL21*extract and feeding solution were prepared by us.

T7 RNAP expression cassette sequence (highlight segment is T7 RNAP gene sequence).

ATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAA
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AATTAACTATGAGAGGATCGCATCACCATCACCATCACGGATCCGCATGCGAGCATGAACACGATTAACATC
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CCCTAAGATGATTGCACGCATCAACGACTGGTTTGAGGAAGTGAAAGCTAAGCGCGGCAAGCGCCCGACAG
CCTTCCAGTTCCTGCAAGAAATCAAGCCGGAAGCCGTAGCGTACATCACCATTAAGACCACTCTGGCTTGCC
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CGACCTGCAGCCAAGCTTAATTAGCTGAGCTTGGACTCCTGTTGATAGATCCAGTAATGACCTCAGAACTCC
ATCTGGATTTGTTCAGAACGCTCGGTTGCCGCCGGGCGTTTTTTATTGGTGAGAATCCAAGCTAGCTTGGCG
AGATTTTCAGGAGCTA

eGFP expression cassette sequence (highlight segment is eGFP gene sequence).

TAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAG
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AACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCG
GATCTGGCGTAATAGCGAAGAGGCCCGC

Synthesis of AuNPs. Synthesis of Au seeds. 100 mL of $HAuCl_4$ (1%) was heated at 80°C in a 250 mL three-necked round-bottomed flask under vigorous stirring. A solution of 1 mg/mL sodium citrate in Milli-Q water (50 mL) was heated to 80°C and then dropped into the three-necked round-bottomed flask quickly. Stirring continued for 2 h. The solution's final color was wine red.

Preparation of AuNPs. We diluted 2.5 mL Au seeds with 60 mL in Milli-Q water, and then mixed them with 10 mL growth solution (0.1% $HAuCl_4$) with vigorous stirring. 0.64 mg/mL L-ascorbic acid solution (20 mL) and 5 mL Sodium citrate (8 mg/mL) were separately dropped into the mixture. Set the mixture for 2 hours

TEM. Morphology of AuNPs were characterized using transmission electron microscopy (TEM). AuNPs and were coated onto carbon-shadowed, formvar 246 coated grids, and examined with a transmission 248 electron microscope (JEM-2100F, Japan).

Synthesis of 2S-branched primers. 2S-branched DNA structure was synthesized in 80 mM NaCl by mix the equimolecular of corresponding oligonucleotide strands (Table 1). To be specific, 10 μL $Y_{a\text{-}S}$ (100 μM), 10 μL $Y_{b\text{-}S}$ (100 μM) and 10 μL T7 RNAP-Y_c or eGFP-Y_c (100 μM) were diluted in 80 mM NaCl aqueous solution to total volume of 100 μL with a final concentration of 10 μM for each oligonucleotideand, then annealed from 95°C to 20°C in less than 2 h. The procedures were performed as following: Denaturation at 95°C for 2 min; Cooling at 65°C for 2 min; Annealing at 60°C for 5.5 min; Further annealing at 60°C for 0.5 min with a continuous temperature decrease at a rate of 1°C per min. The final annealed products were stored at 20°C. The finial concentration of synthesized 2S-branched DNA was 10 μM. Then the synthesized samples were cross-linked being incubated with 4',5',8'-trimethypsoralen at a molar ratio of 1:1 between 4',5',8'-trimethypsoralen and DNA

base pair. The mixture was diluted with aqueous NaCl solution (40 mM) and exposed to UV light for cross-linking using a 365 nm UV-A lamp (Scientz03-II, SCIENTZ BIOTECHNOLOGYCO) of approximately 4.0 J on the ice. The thermostability of branched primers was confirmed with 8% Ready Gel TBE-urea polyacrylamide denaturing gel, and almost 100% 2S-branched DNA was cross-linked after crosslinking treatment.

Melting curve analysis. Melting curves were determined as our previous method[1]. In general, they were determined by measureing the ultraviolet absorption of 2.0 μM 2S-branched DNA or 2S-branched primer at 260 nm with gradually heating up temperature from 25°C to 95°C. In particular, 2S-branched DNA or 2S-branched primer was diluted in aqueous NaCl solution (48 mM) with a final concentration of 2 μM. 500 μL sample was placed into the cuvette (45×12.5×4.5 mm), then the ultraviolet absorption of samples at 260 nm was measured every 2°C increase with heating up temperature from 25°C to 95°C by UV-VIS-NIR spectrometer (Shimadzu UV-3600, Japan)

Preparation of *E. coli BL21* extracts. We prepared *E. coli BL21* extracts based on our previously reported method[2]. *E. coli BL21* strains initial cultivation was performed in 20 mL of LB medium (16 g/L tryptone, 10 g/L yeast extract, 5 g/L NaCl) at 37°C in an orbital shaker at 250 rpm. After 12 hours incubation, 2 mL of starter culture was used to inoculate 1 L fresh LB medium, which contained YTPG salts (2 mM NaH_2PO_4, 4 mM Na_2HPO_4) and 0.1 M glucose, and incubate on shaker (250 rpm) at 37°C. When the OD_{600} of grow culture reached 1.8, collected the cell pellets by centrifugation with high speed refrigerated centrifuge (GL-21M, CENCE) at 5500 rpm and 4°C for 10 min. Pellets was thoroughly dissolved in ice-cold 20% sucrose solution and incubated on ice for 10 min. Resuspended cells were transferred to a weighed JA20 container and collected by centrifugation at 6800 rpm and 4°C for 10 min. Cell pellets were then washed four times with ice-cold MQ. After the final wash and centrifugation, the pelleted cells were resuspended in lysate buffer (10 mM Tris, 60 mM potassium glutamate, 14 mM magnesium glutamate, 1 mM DTT), and 1 g pellets were resuspended by 1.3 mL lysate buffer. The smooth suspended cells were shaken or knocked by hand and disrupted by Noise Isolating Chamber (SCIENTZ, China). The lysate was then centrifuged twice at 15700 rpm and 4°C for 30 min. The resultant supernatant was collected as cell lysate, and then immediately flashed frozen in liquid nitrogen and stored at -80°C.

Equation S1: Spatial distance between two genes in free gene circuit system

$$\mathrm{d} = \sqrt[3]{\frac{1}{cN_A}}$$

Where c is the concentration of total genes, N_A is Avogadro's constant.

Oligonucleotides

Table S1. Oligonucleotides used for synthesis of branched primers and PCR.

Strand	Sequence	Use
$Y_{a\text{-}S}$	5'TGGACCGATATCATATGTACATTACTATAGTACCTGAGSH3'	Building blocks of branched primer. Aquamarine part is T7 RNAP reverse primers for PCR, and orange part is eGFP forward primers for PCR.
$Y_{b\text{-}S}$	5' CTCAGGTACTATAGTAATGATCATCTATAGTACAGCCTSH3'	
T7 RNAP-Y_c	5'AGGCTGTACTATAGATGATTACATATGATATCGGTCCATAG CTCCTGAAAATCTCGCCAAGC3'	
eGFP-Y_c	5'AGGCTGTACTATAGATGATTACATATGATATCGGTCCATAA CCGTATTACCGCCTTTGAGT3'	
T7 RNAP forward primer	5'ATAGGGGTTCCGCGCACATTTCC3'	Forward primer for PCR to generate 2S-T7 RNAP gene
eGFP reverse primer	5'GCGGGCCTCTTCGCTATTA3'	Reverse primer for PCR to generate 2S-eGFP gene

RESULTS AND DISCUSSION

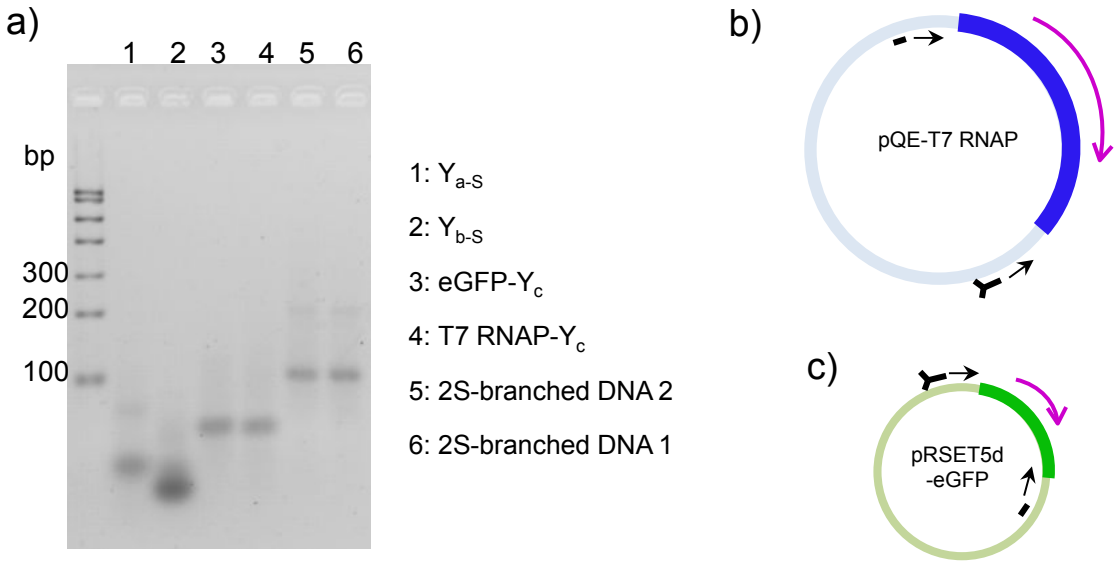
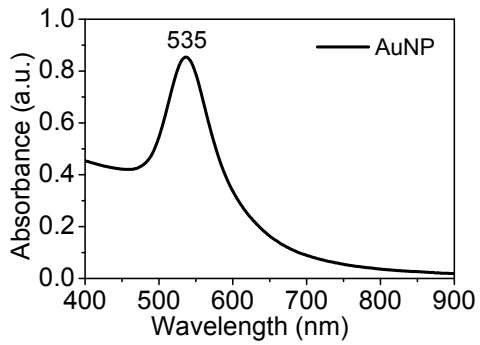


Figure S1. Verification of the formation of 2S-branched DNA and schemes of synthesizing 2S-genes from plasmids. (a) 3% agarose gel electrophoresis of ssDNA (Y_{a-S}, Y_{b-S}, eGFP-Y_c and T7 RNAP-Y_c) and 2S-branched DNA (2S-branched DNA 1 and 2S-branched DNA 2). (b) Scheme of synthesizing 2S-T7 RNAP gene from pQE-T7 RNAP plasmid by 2S-branched reverse primer and linear forward primer. (c) Scheme of synthesizing 2S-eGFP gene from pRset5d-eGFP plasmid by 2S-branched forward primer and linear reverse primer.

a)



b)

Diameter (nm)	50.00±0.113
Zeta (mA)	-34.57±0.386

c)

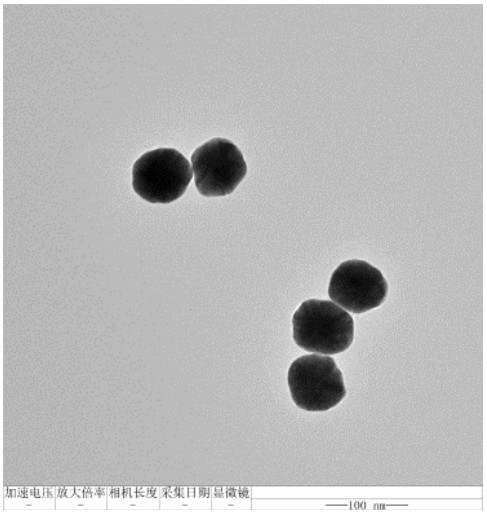


Figure S2. Characterizations of the synthesized AuNPs. (a) UV-Vis absorption spectrum of AuNPs. (b) Particle size and surface potential analysis of AuNPs. (c) TEM image of AuNPs.

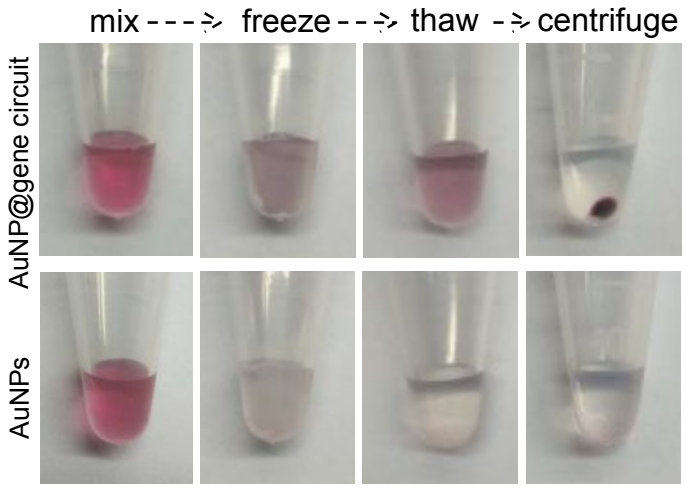


Figure S3. Synthesis of AuNP@gene circuit nanostructure using freezing method. Photographs showing the synthesis process of AuNP@gene circuit. The gene functionalized AuNPs were still red and well dispersed after freezing, and the sample easily precipitated after centrifugation. As control, free AuNPs freezing resulted in the colour change from red to colourless.

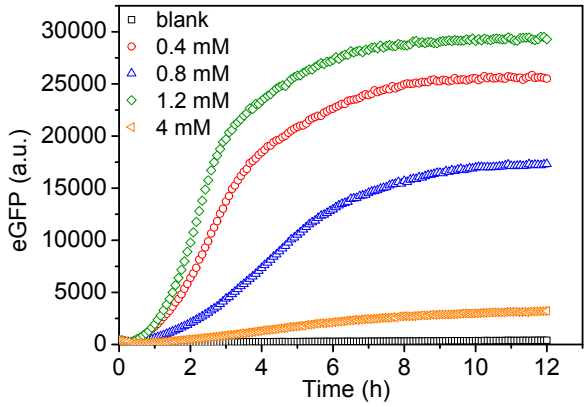


Figure S4. eGFP expression from free gene circuit with different concentrations of IPTG in cell-free system.

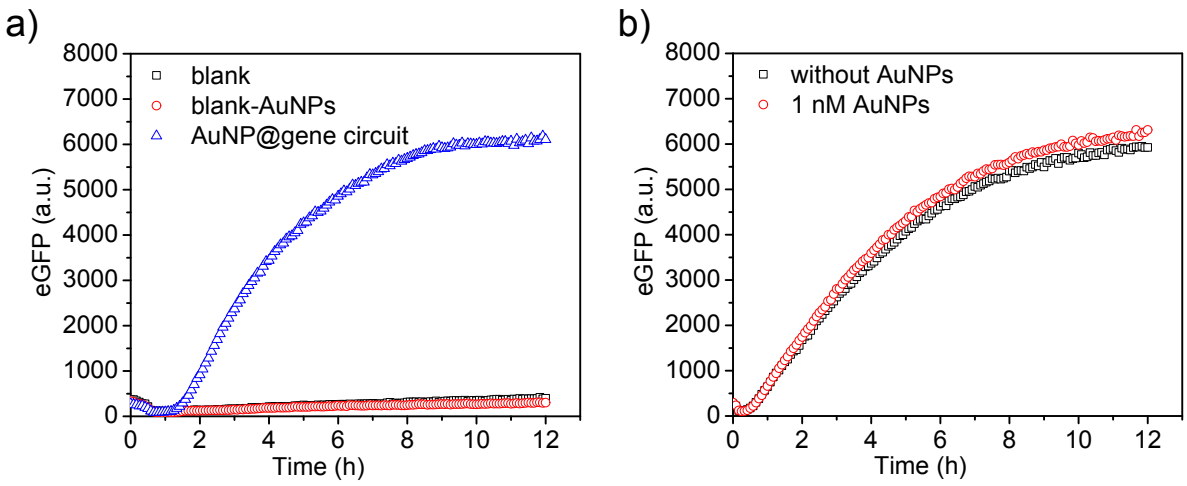


Figure S5. a) Free AuNPs have no influence in the background fluorescence value of cell-free system. b) 1 nM free AuNPs have no influence in protein expression of cell-free system.

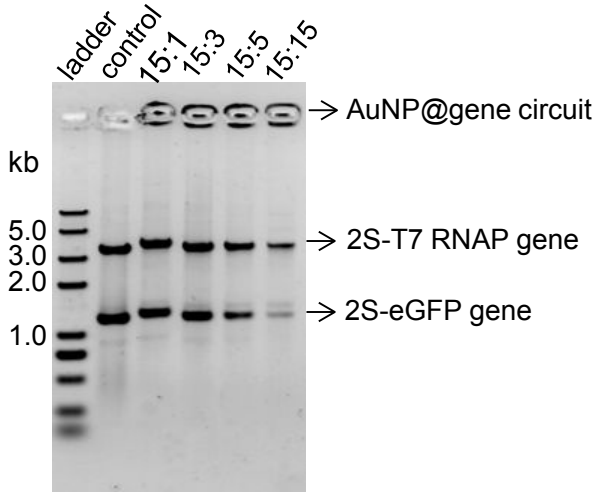


Figure S6. Agarose gel electrophoresis of AuNP@gene circuit with the stoichiometric ratio of 2S-eGFP genes to AuNPs variying from 15:1, 15:3, 15:5 to 15:15. The bounding efficiencies of genes in these four systems were 29.94%, 33.06%, 51.13% and 79.27%, respectively.

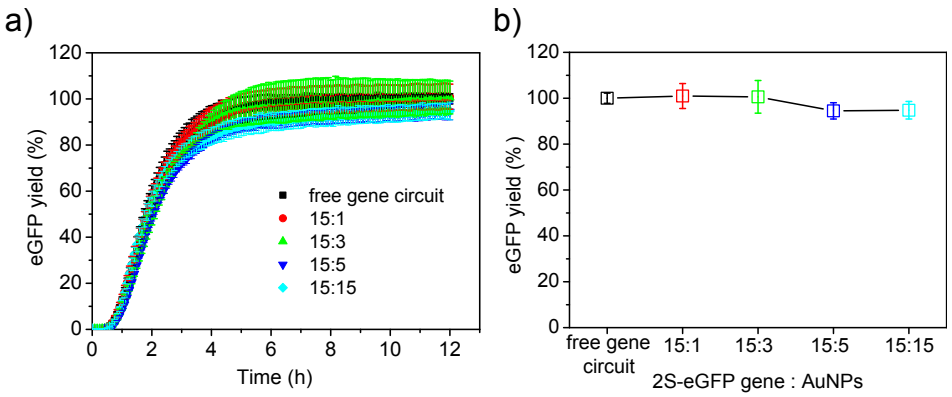


Figure S7. eGFP expression kinetics (a) and eGFP yields (b) with conjugating 2S-eGFP genes and 2S-T7 RNAP genes to AuNPs separately, and the stoichiometric ratios of 2S-eGFP genes (or 2S-T7 RNAP genes) to AuNPs were varied from 15:1, 15:3, 15:5 and 15:15. The concentrations of 2S-eGFP genes and 2S-T7 RNAP genes were 1 nM.

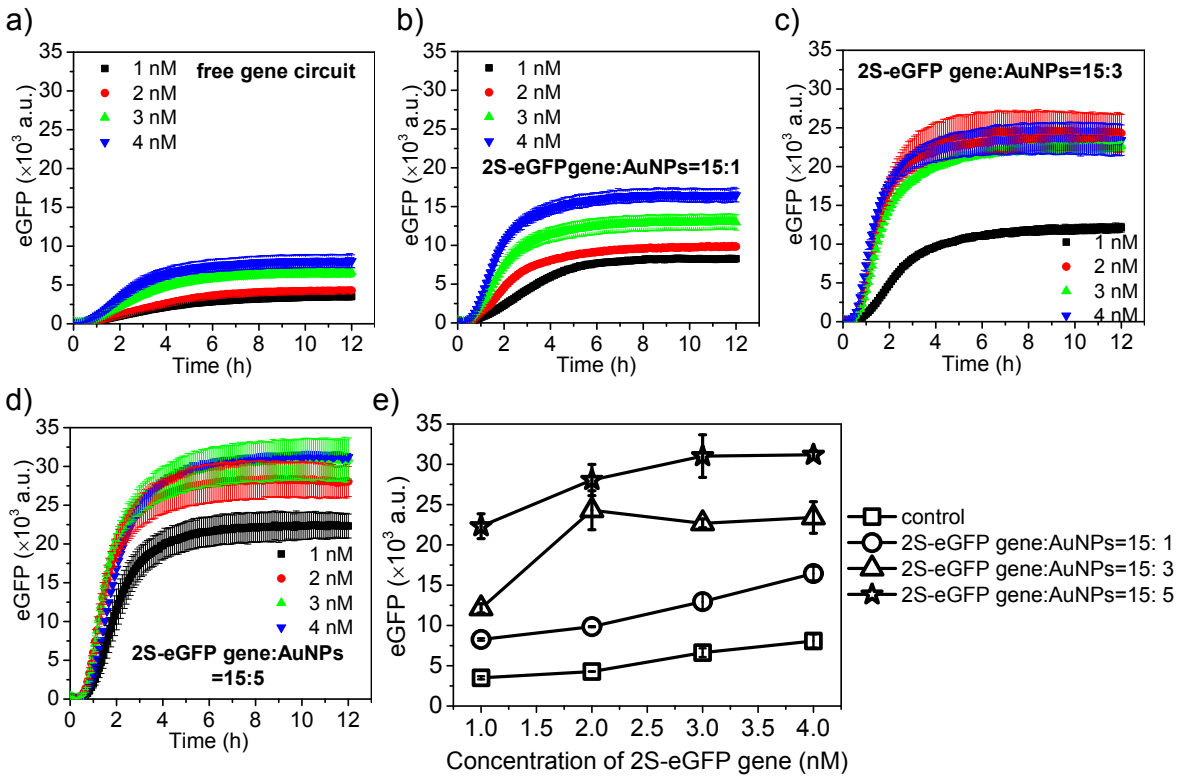


Figure S8. The effect of gene compartment on cascade gene expression with different concentration of genes. The eGFP expression with varying the concentration of 2S-eGFP gene (or 2S-T7 RNAP gene) from 1.0 to 4.0 nM in (a) free gene circuit system and (b-d) three gene compartment systems with the fixing the stoichiometric ratio of 2S-eGFP genes to AuNPs at (b) 15:1, (c) 15:3 and (d) 15:5, respectively. (e) eGFP yields in different gene compartment systems after expressing for 12 h. Error bars represent standard deviations from three replicates.

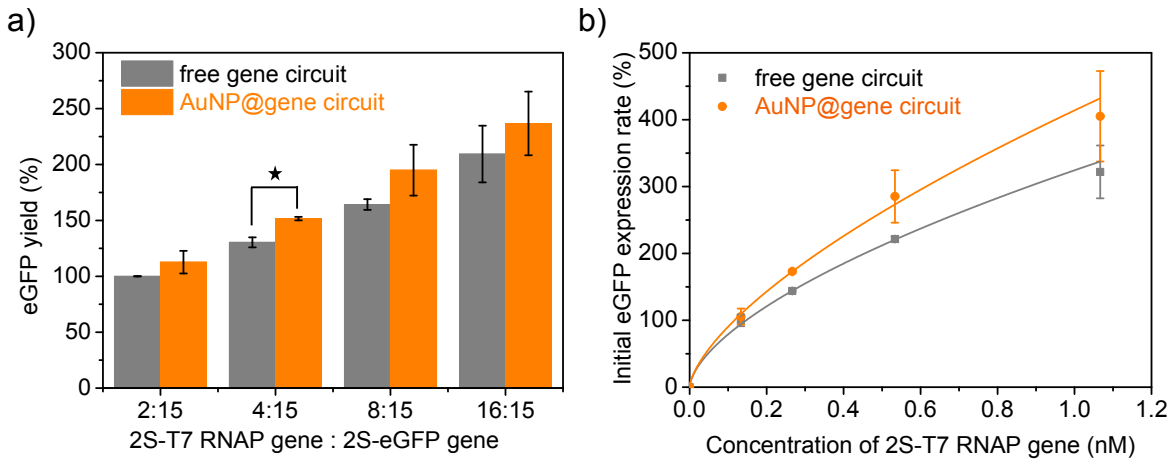


Figure S9. a) eGFP yield of different gene circuit compartment systems and free gene circuit systems with varying the stoichiometric of 2S-T7 RNAP genes to 2S-eGFP genes from 2:15, 4:15, 8:15, 15:15 to 16:15. The molar ratio of 2S-eGFP gene to AuNPs was set as 15:5, and the concentration of 2S-eGFP genes was kept at 1

nM. b). Experimental data (dots) and fitting curves (lines) of the initial eGFP expression rate as a function of the added concentration of 2S T7-RNAP genes. Error bars represent standard deviations from three replicates. ★: $P<0.05$.

REFERENCES

(1) Guo, X.; Bai, L.; Li, F.; Huck, W. T. S.; Yang, D. Branched DNA architectures produced by pcr-based assembly as gene compartments for cell-free gene-expression reactions. *ChemBioChem* **2019**, *20*, 2597-2603.
(2) Yang, D.; Peng, S.; Hartman, M. R.; Gupton-Campolongo, T.; Rice, E. J.; Chang, A. K.; Zi, G.; Lu, G. Q.; Dan, L. Enhanced transcription and translation in clay hydrogel and implications for early life evolution. *Sci. Rep.* **2013**, *3*, 3165-3170.