Supporting information

Negative Feedback Regulation of Fatty Acid Production Based on a Malonyl-CoA Sensor-Actuator

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Figure S1. Growth inhibition from the acetyl-CoA carboxylase overexpression. SAcc1 and SAcc2 strains (Table 2) were cultured in minimal medium with 2% glucose and cell growth was monitored continuously in a plate reader until stationary phase was reached. Cell growth at 20 hours after induction was plotted against different IPTG induction levels.

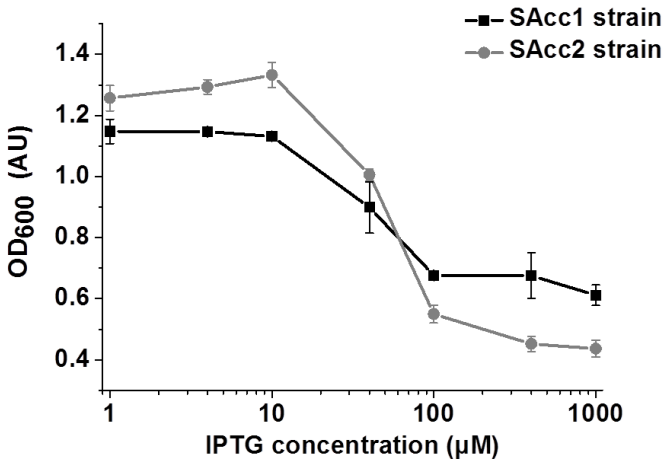


Figure S2. Comparison of FA2 and FA3 cell growth under various IPTG induction levels. FA2 and FA3 strains were cultured in minimal medium with 2% glucose and 0.01% arabinose, and were induced with 200 nM aTc and a) 0 μM, b) 10 μM, c) 40 μM, d) 100 μM, e) 400 μM, f) 1000 μM IPTG. Cell growth was monitored continuously on a plate reader (See Method). g) OD_{600} of FA2 and FA3 strains at 8 hours post induction.

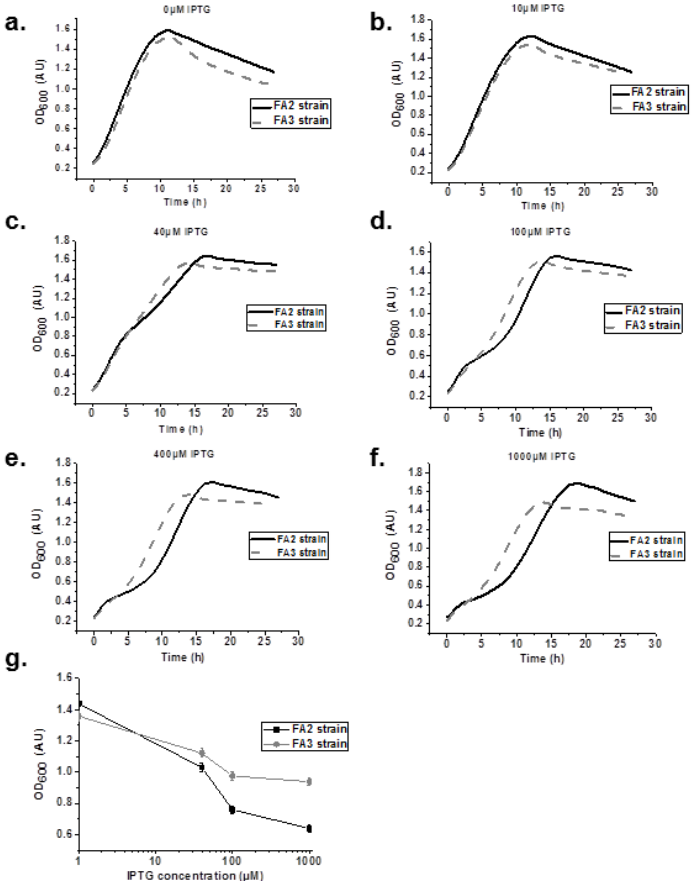


Figure S3. Effect of negative feedback circuit on cell growth. All the strains were cultured in minimal medium with 2% glucose and 0.01% arabinose, and were induced with 200 nM aTc and 0.1 mM of IPTG. Cell growth was monitored continuously on a plate reader.

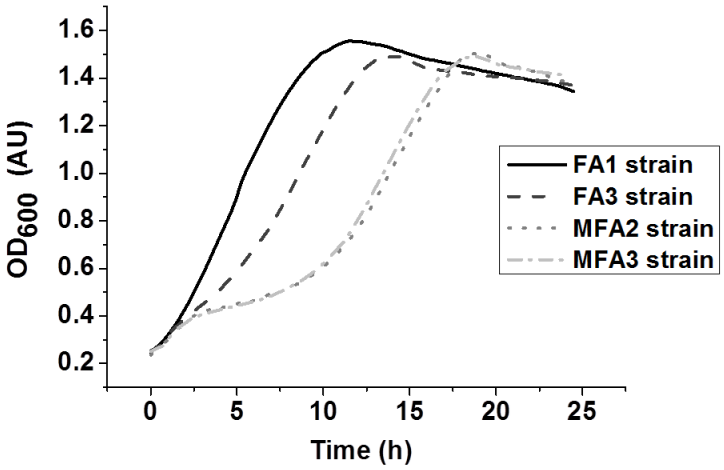
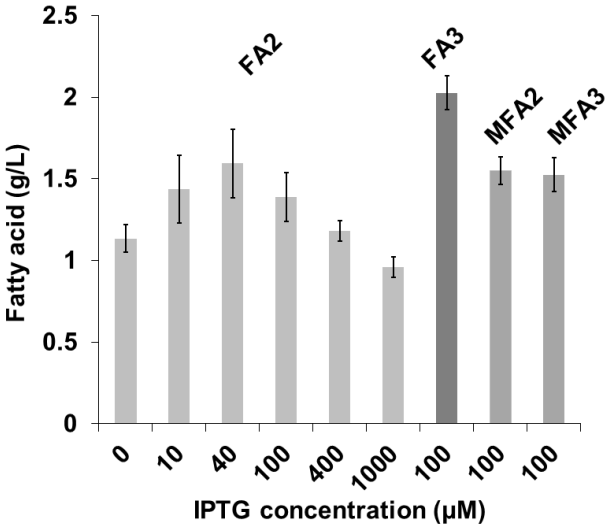


Figure S4. Comparison of fatty acid production of the FA2, FA3, MFA2 and MFA3 strains. FA2 strain was induced with 200 nM aTc and various amounts of IPTG, and cultured in minimal medium with 2% glucose and 0.01% arabinose for 25 hours. FA3, MFA2 and MFA3 strains were cultured under the same condition and induced with 200 nM aTc and 0.1 mM IPTG induction. Fatty acid titers were analyzed as described in Methods.



Mathematical model of Malonyl-CoA inducible System

At equilibrium, malonyl-CoA binding to FapR is described by the equation (1)

$$FapR_f = \frac{K_d}{Mal+K_d} FapR_t \quad (1)$$

where $FapR_f$ is the concentration of free FapR, Mal is the concentration of free malonyl-CoA, K_d is the dissociation constant of malonyl-CoA binding to FapR, $FapR_t$ is the total intracellular concentration of FapR.

The activity of a malonyl-CoA-responsive promoter P_{mal}, as represented by the normalized cell culture fluorescence, can be described by equation (2) (based on previously established models[1, 2]):

$$P_{mal} = P_{max} \frac{K_p P}{1+K_p P+2K_1 FapR_f+{K_1}^2 {FapR_f}^2} \quad (2)$$

where P_{max} is the fully activated promoter activity (represented by the normalized cell culture fluorescence in the absence of the repressor). K_p is the binding constant of the RNA polymerase to the promoter, and P is the concentration of the RNA polymerase. K_1 is the binding constant of FapR to the promoter.

Combining equations (1) and (2):

$$P_{mal} = P_{max} \frac{K_p P}{1+K_p P+2K_1 \frac{K_d}{Mal+K_d} FapR_t+{K_1}^2 (\frac{K_d}{Mal+K_d} FapR_t)^2} \quad (3)$$

Equation (3) was fitted to the fluorescence/malonyl-CoA data using malonyl-CoA concentrations quantified from LC-MS and the normalized cell culture fluorescence measured from plate-reader at various induction levels. Parameters were fitted using Matlab.

References

1. Moon, T. S., Lou, C. B., Tamsir, A., Stanton, B. C., and Voigt, C. A. (2012) Genetic programs constructed from layered logic gates in single cells, *Nature 491*, 249-253.
2. Bintu, L., Buchler, N. E., Garcia, H. G., Gerland, U., Hwa, T., Kondev, J., and Phillips, R. (2005) Transcriptional regulation by the numbers: models, *Curr Opin Genet Dev 15*, 116-124.