

## Synthetic genetic circuits for dynamic fine-tuning of metabolic modules in *Bacillus subtilis*

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### **Abstract**

Dynamic regulation is an effective strategy for fine-tuning metabolic pathways in order to maximize target product synthesis. However, achieving dynamic and autonomous up- and down-regulation of the metabolic modules of interest simultaneously, still remains a great challenge. In this work, we created an autonomous dual-control (ADC) system, by combining CRISPRi-based NOT gates with novel biosensors of a key metabolite in the pathway of interest. By sensing the levels of the intermediate glucosamine-6-phosphate (GlcN6P) and self-adjusting the expression levels of the target genes accordingly with the GlcN6P biosensor and ADC system enabled feedback circuits, the metabolic flux towards the production of the high value nutraceutical N-acetylglucosamine (GlcNAc) could be balanced and optimized in *Bacillus subtilis*. As a result, the GlcNAc titer in a 15-L fed-batch bioreactor increased from 59.9 to 131.6 g/L, which is the highest titer ever reported of this valuable molecule, indicating the robustness and stability of the synthetic circuits in a large bioreactor system. Remarkably, this self-regulatory methodology does not require any external level of control such as the use of inducer molecules or switching fermentation/environmental conditions. Moreover, the proposed programmable genetic circuits may be expanded to engineer other microbial cells and metabolic pathways.

### **Brief Biography**

Dr. Long Liu is a Professor at the School of Biotechnology, Jiangnan University, China. His research interests focus on the development of *Bacillus subtilis* chassis for the synthesis of functional nutraceuticals, including mono- and oligo-saccharides, small molecular organic acids and fat-soluble vitamins. He has published over 100 peer-reviewed research and review papers in journals such as *Nature Communications*, *Trends in Biotechnology*, *Biotechnology Advances*, *Metabolic Engineering*, *ACS Synthetic Biology*, and *Biotechnology and Bioengineering*. Currently he is an editorial board member of *Microbial Cell Factories*, *Metabolic Engineering Communications*, *Frontiers in Bioengineering and Biotechnology*, *Biotechnology Notes* and *Scientific Reports*. He is also invited as the guest editor of several special issues for the journals including *Bioresource Technology*, *Microbial Cell Factories*, and *Biotechnology and Applied Biochemistry*.

### **Brief CV**

**Long Liu, Ph.D.**

School of Biotechnology, Jiangnan University

**Education:**

B.S Biotechnology, Yantai University, China, 2004

Ph.D. Fermentation Engineering, Jiangnan University, China, 2009

**Professional Career:**

2009.05-2010.09: Jiangnan University, China, Assistant Professor.

2010.10-2014.09: Jiangnan University, China, Associate Professor.

2012.03-2013.03: Georgia Institute of Technology, US, Postdoctoral Fellow.

2014.10-Present: Jiangnan University, China, Professor.

**Research Interests:**

1. Microbial metabolic engineering
2. Microbial Systems and Synthetic Biology
3. Microbial cell factories for nutraceuticals

**Selected publications**

1. Deng, J. et al. *Metabolic Engineering*, 2019, 55: 179-190.
2. Gu, Y. et al. *Metabolic Engineering*, 2019, 51: 59-69.
3. Wu, Y. et al. *Metabolic Engineering*, 2018, 49: 232-241.
4. Gu, Y. et al. *Metabolic Engineering*, 2018, 50: 109-121.
5. Cui, S. et al. *ACS Synthetic Biology*, 2019, 8: 1826-1837.
6. Deng, C. et al. *Biotechnology and Bioengineering*, 2019, 116: 5-18.
7. Liu, J. et al. *ACS Synthetic Biology*, 2018, 7: 2139-2147.
8. Niu, T. et al. *ACS Synthetic Biology*, 2018, 7: 2423-2435.
9. Guan, N. et al. *Biotechnology and Bioengineering*, 2018, 115: 483-494.
10. Liu, Y. et al. *Nature Communications*, 2016, 7: 11933.