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Molecular tools to engineer cyanobacteria for industrial biotechnology

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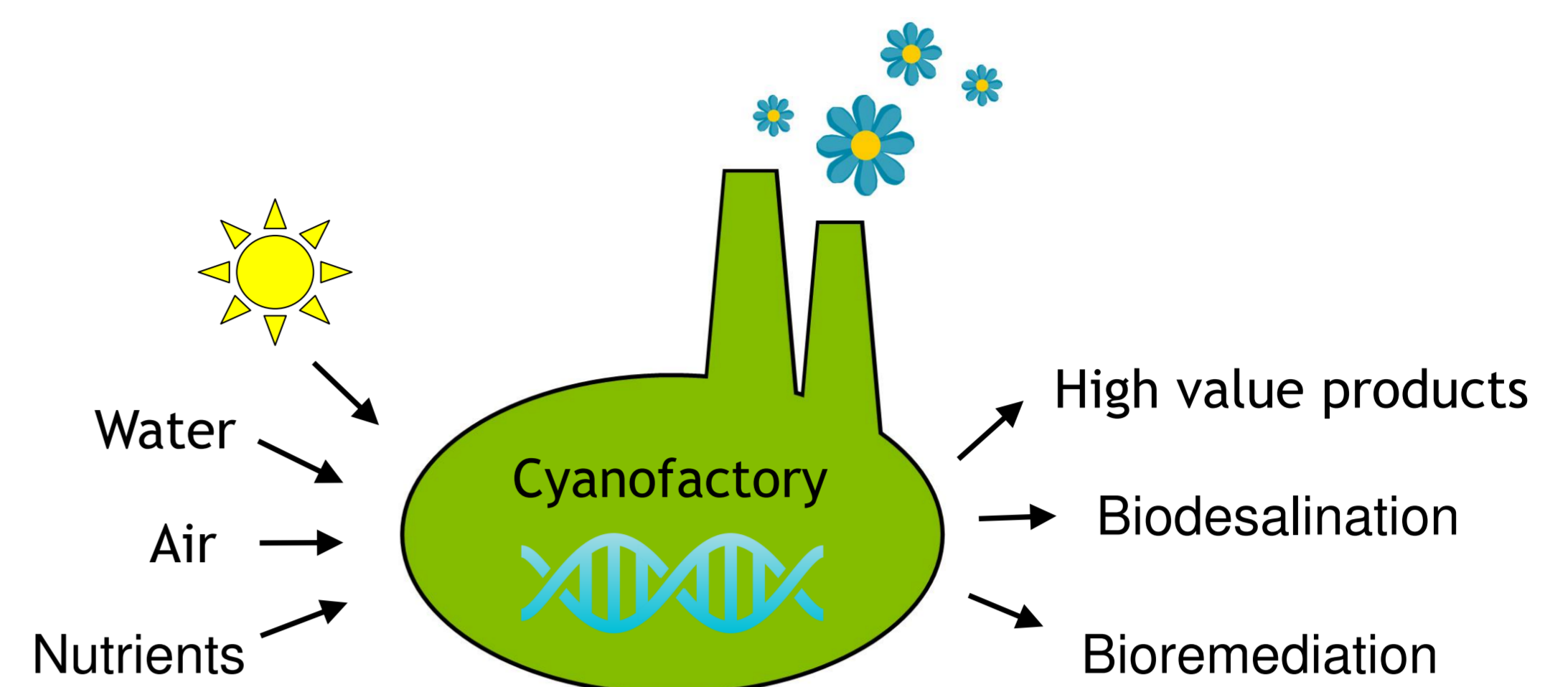
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Cyanobacteria as a sustainable chassis

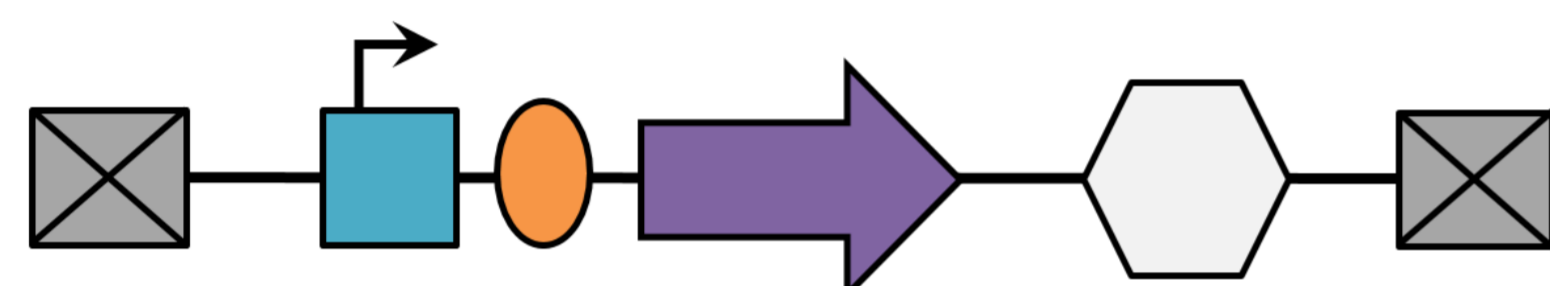
- Prokaryotes - simple organisms, rapid growth, small genomes, easily transformed
- Photosynthetic - minimal input, therefore cheap feedstock and lower risk of contamination
- Extremely diverse - habitat, morphology, metabolism, wide range of natural products

...but the engineering toolbox is still limited



Introducing foreign DNA

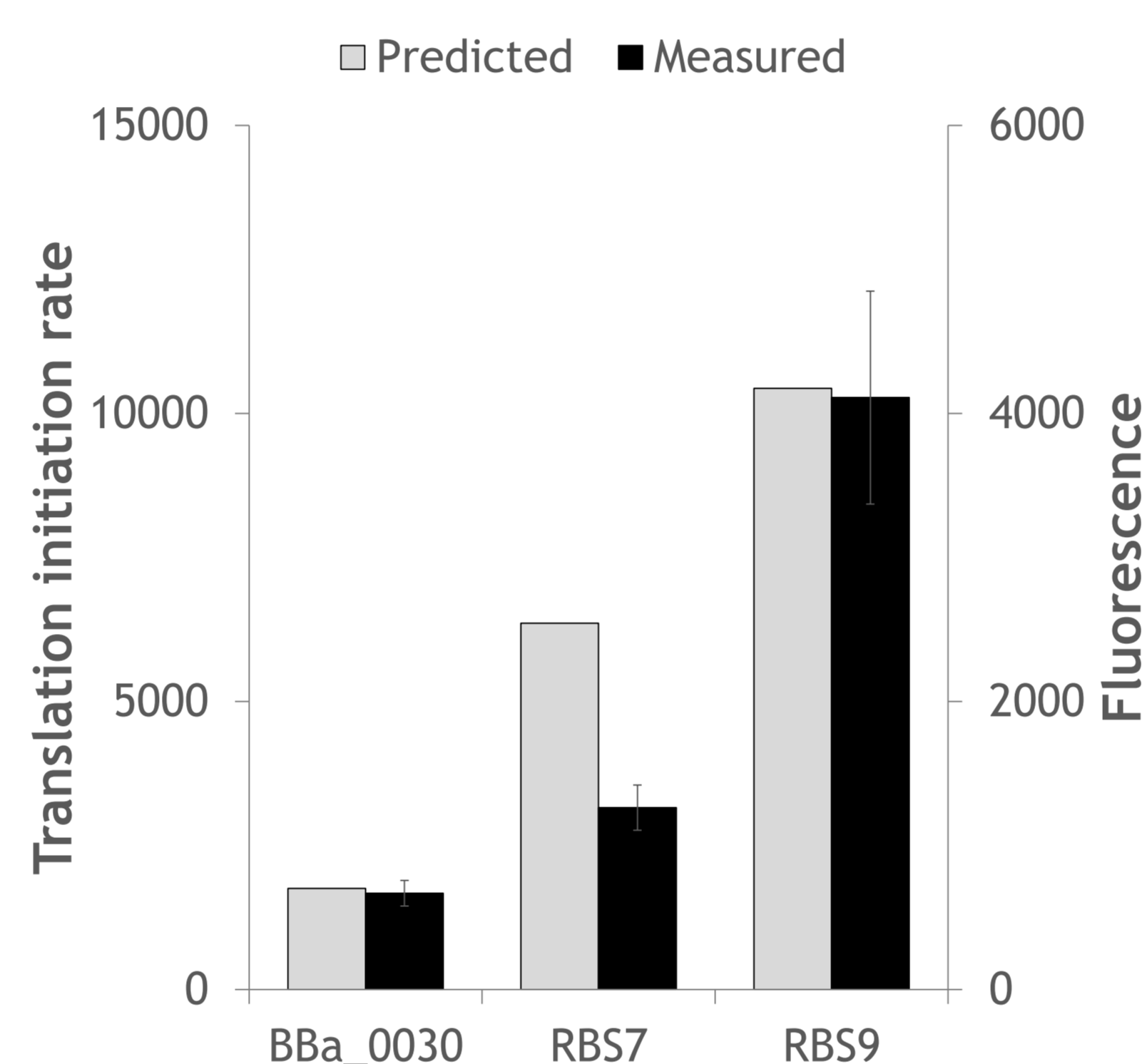
- Plasmid vectors for stable integration to neutral sites in the genome
- BioBrick compatible



- ⊠ Homologous recombination sites
- Promoter
- Ribosome binding site
- Protein coding sequence
- ⬡ Selectable marker

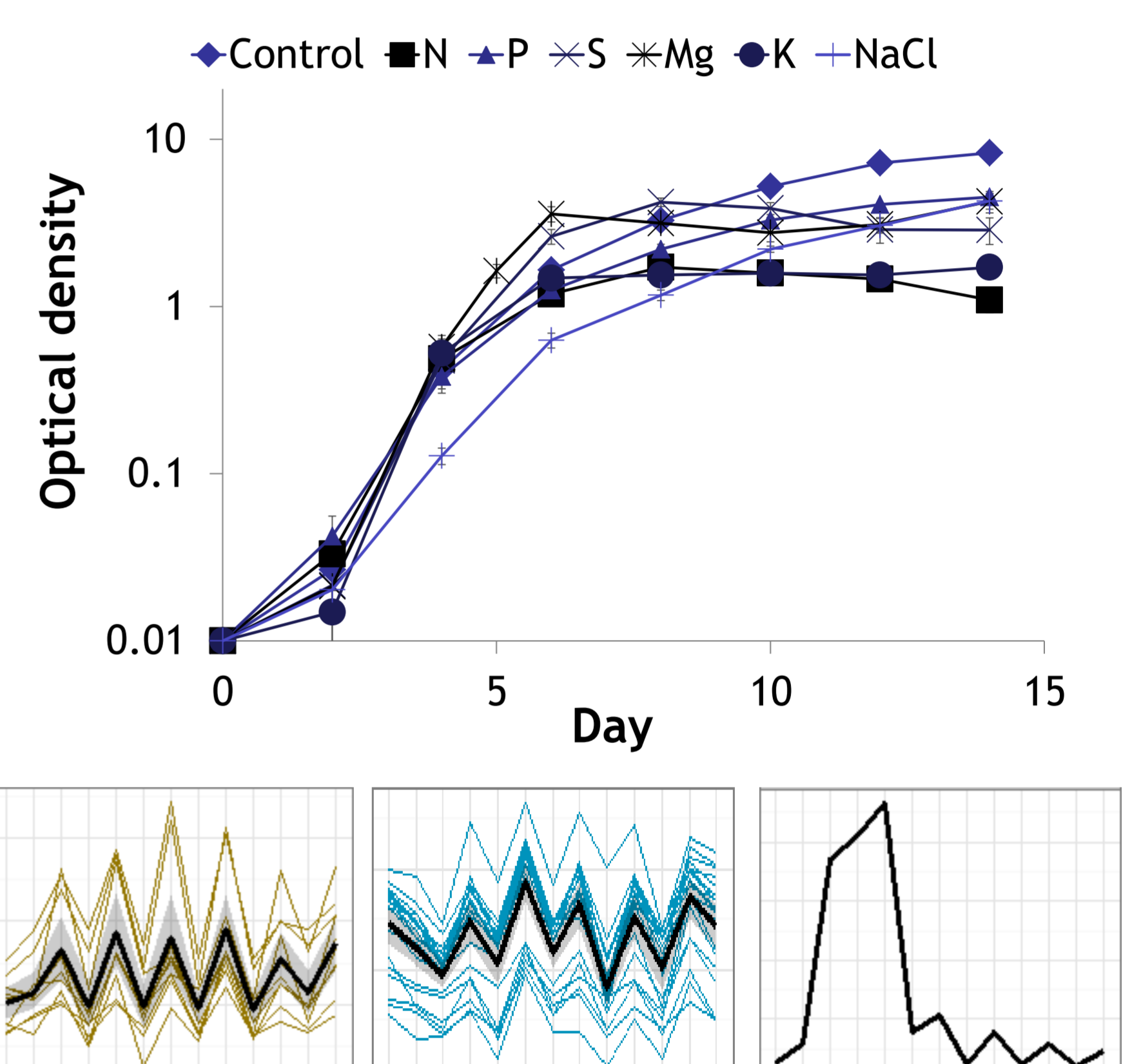
Controlling translation initiation

- Forward engineering of synthetic ribosome binding sites based on *in silico* calculators



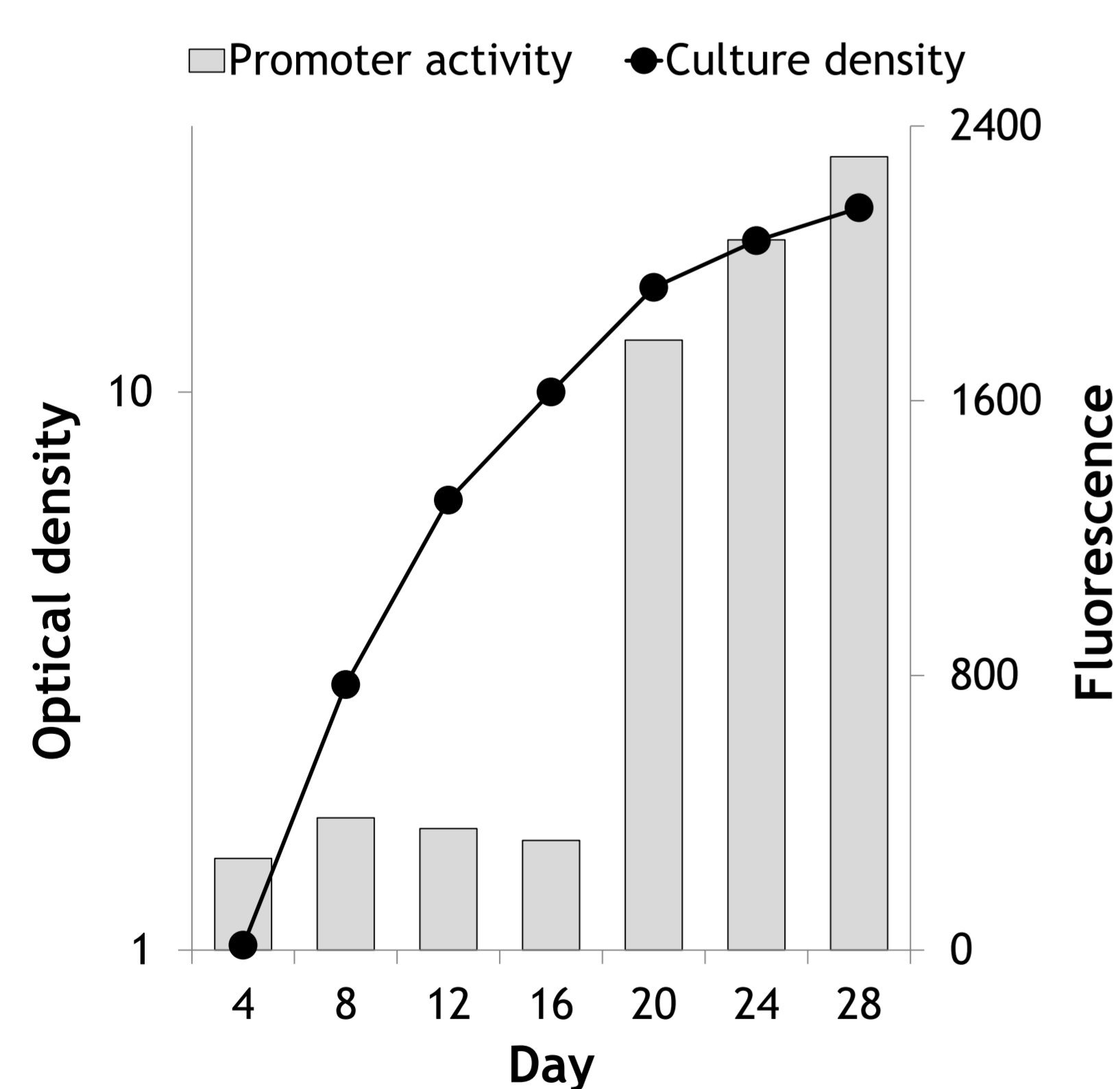
Controlling growth kinetics

- Defined conditions, extensive RNAseq analysis



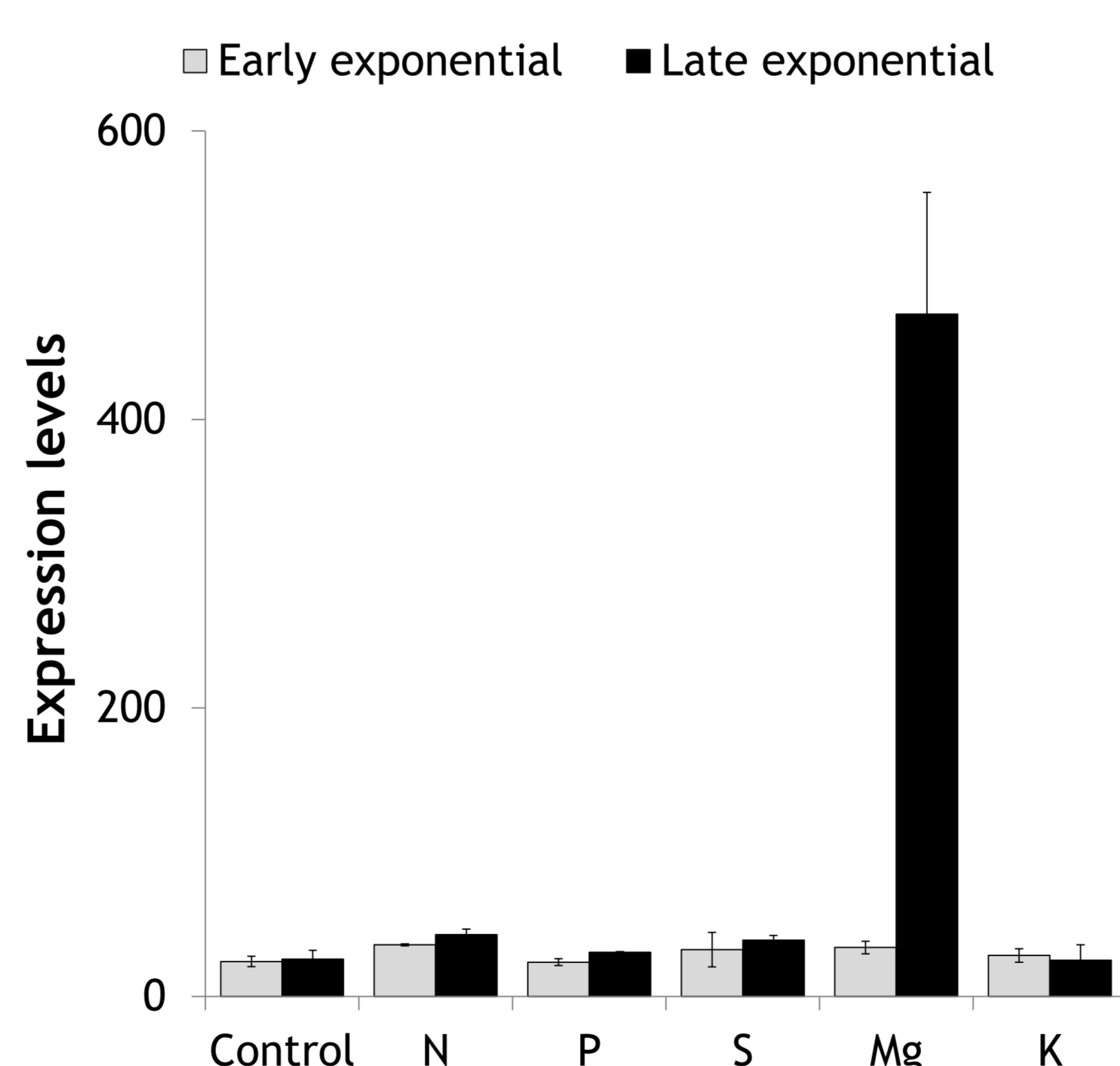
Growth-responsive transcription

- Induced in the late exponential phase



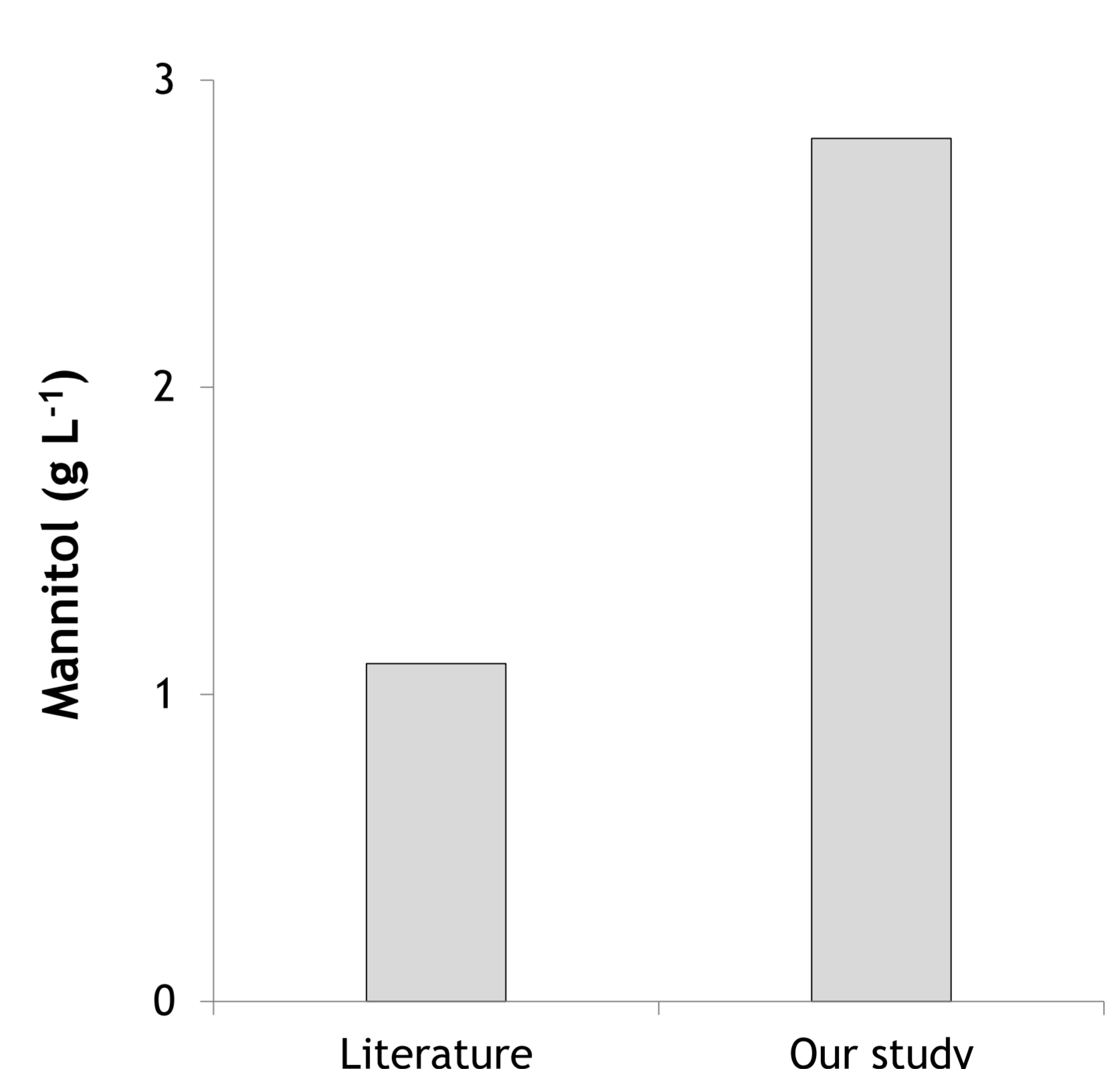
Nutrient-responsive transcription

- Induced under specific nutrient limitations



Enhanced product synthesis

- Better tools make for better titres



Summary

- A comprehensive toolkit has been established for rapid, rational design of cyanobacteria
- Control at various levels of the production process: culture growth, transcription, translation and ultimately product synthesis
- Novel conditions to modulate growth kinetics identified and transcriptomic responses analysed
- Novel growth phase- and nutrient-responsive promoters developed

