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Unraveling plant–microbe symbioses using single-cell and spatial transcriptomics

Background/Objective

Within symbioses, the development of novel microbial and plant structures is driven by symbiosis-specific gene expression in a few specific cell populations.

Approach

We reviewed the literature for recent advances in using these transcriptomic techniques to study plant-microbe symbioses

Results

Single-cell and spatial RNA-seq are powerful tools to study plant–microbe endosymbiosis, can facilitate the identification of genes, and can improve our general understanding of the complex biology of symbiotic interactions.

Significance/Impacts

- Beneficial plant–microbe interactions are critical to plant productivity in both natural and agricultural ecosystems.
- Genetic engineering to take better advantage of these interactions relies on the identification of symbiosis-responsive plant and microbial genes that can serve as targets for modification.

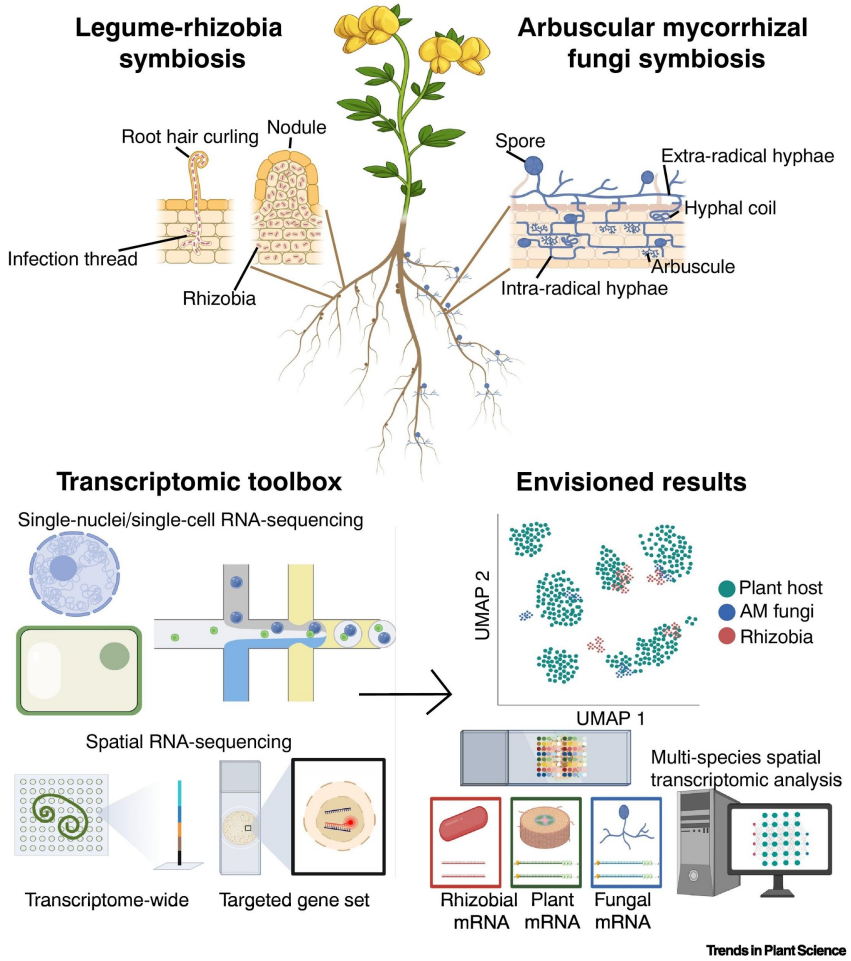

Figure. Transcriptomics as a tool for plant–microbe endosymbioses

Serrano K., et. al. Trends in Plant Science. doi: 10.1016/j.tplants.2024.06.008

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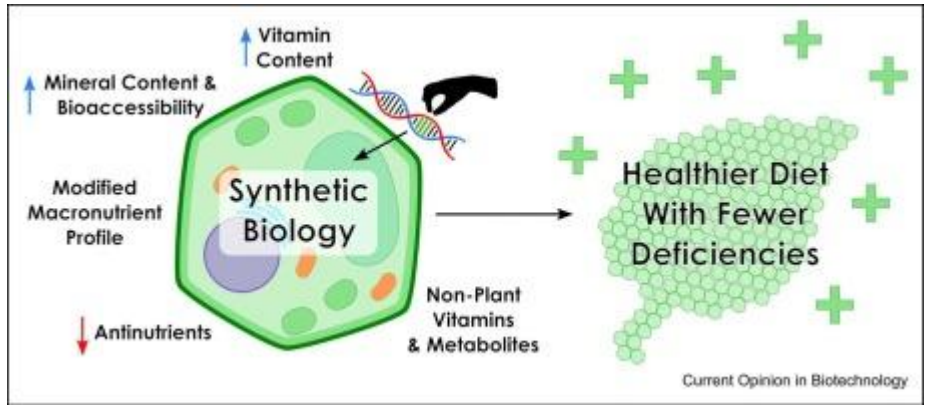
Plant synthetic biology as a tool to help eliminate hidden hunger

Background/Objective

Improving plant nutritional content through biotechnology techniques such as synthetic biology is a promising strategy to help combat hidden hunger caused by the lack of affordable and healthy foods in human diets



Approach

We reviewed recent biotechnological approaches to biofortifying plants with vitamins, minerals, and other metabolites, and summarise synthetic biology advances that offer the opportunity to build on these early biofortification efforts.

Results

- Genetic modification of plants for nutritional benefits is now commonplace.
- Vitamin accumulation by genetic modification has been widely explored.
- Other key targets include reducing antinutrients and tuning macronutrient profiles.

Significance/Impacts

- Changing regulations may see more genetically modified plants approved for use.
- Future studies need to address target bioaccessibility and real-world validation.

Edwards R.A., et. al. Current opinion in biotechnology. doi: 10.1016/j.copbio.2024.103168

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Defined synthetic microbial communities colonize and benefit field-grown sorghum

Background/Objective

- The rhizosphere constitutes a dynamic interface between plant hosts and their associated microbial communities.
- Despite the potential for enhancing plant fitness by manipulating the rhizosphere, the engineering of the rhizosphere microbiome through inoculation has posed significant challenges.

Approach

We assembled a synthetic rhizosphere community ('SynCom') with 57 bacterial strains to investigate its stability and effect on growth of *Sorghum bicolor*.

Results

The SynCom effectively promoted the growth of sorghum both above and below ground in both laboratory and native field contexts.

Significance/Impacts

The results underscore the potential utility of SynComs for modulating crop performance in controlled and native environments alike.

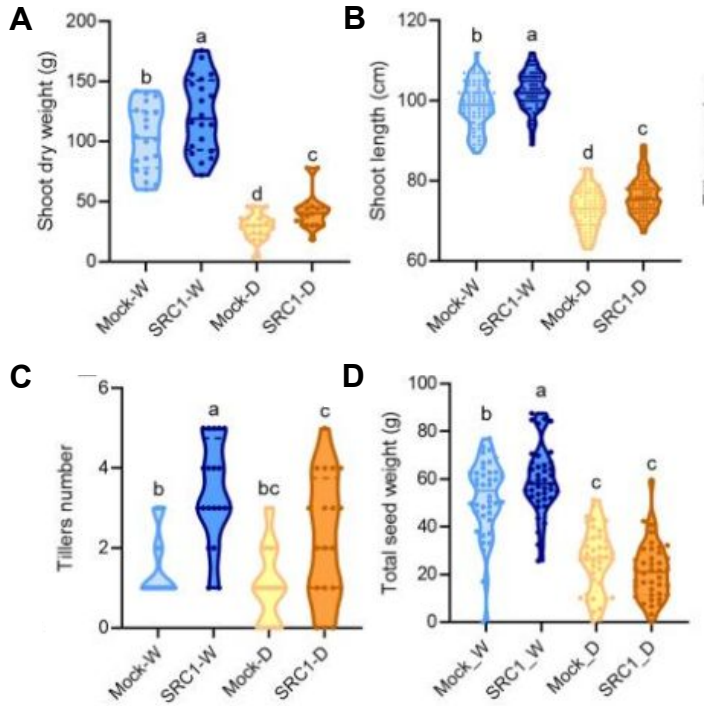

Figure. Sorghum plants inoculated with the 57-member SynCom in the field had increased root and shoot biomass. Tiller numbers were also increased and seed weight was increased under well watered conditions but not under drought. W- well watered, D - drought. Biomass was determined with 8-week-old plants

Fonseca-García C., et. al. The ISME journal. doi: 10.1093/ismejo/wrae126

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Statistical Design of Experiments guided high titer microbial production of a flavor compound

Background/Objective

Demonstrate sustainable production of the flavor compound 2,3,5,6-Tetramethylpyrazine (TMP) at high titer in an industrially relevant host.

Approach

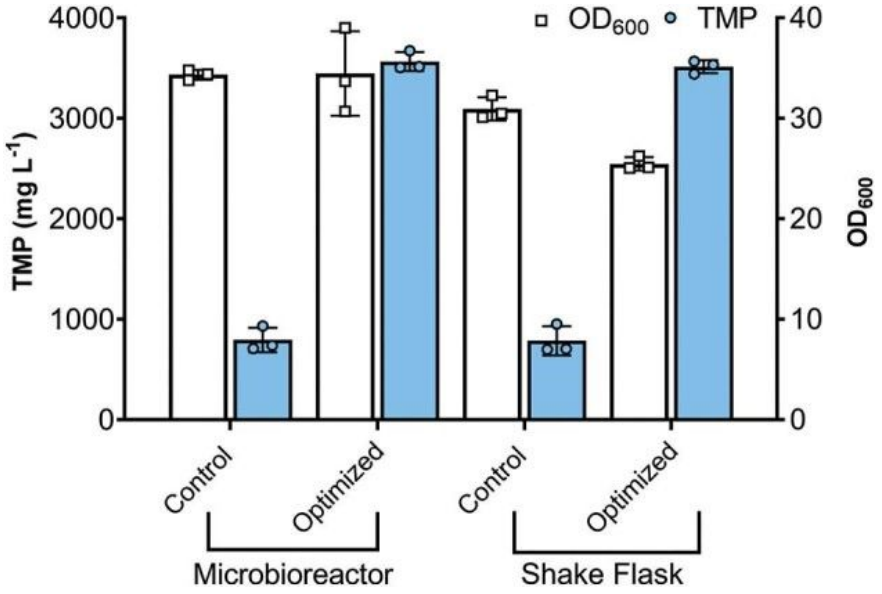
Metabolic engineering of *Corynebacterium glutamicum* followed by post-strain engineering medium optimization using a statistical approach to identify components affecting TMP production.

Results

- An engineered *C. glutamicum* strain reliably producing TMP.
- The engineered strain grew robustly and produced TMP on real world carbon streams.
- Glucose (carbon source) and Urea (one of the nitrogen source) were most critical parameters for high titer production.

Significance/Impacts

- Bio-based production of pyrazines and its precursors (commodity chemicals).
- Data from classical optimization here serve as test-sets for further machine learning based optimization (such as ART).



Enhanced 2,3,5,6-Tetramethylpyrazine (TMP) production (blue bars) in the engineered *Corynebacterium glutamicum* strain growing on the optimized (guided by statistical tools) CGXII minimal medium in different formats (high-throughput microbioreactor and shake flasks)

Srinivasan et al (2024) JIMB; 10.1093/jimb/kuae026

ABPDU
ADVANCED BIOFUELS AND BIOPRODUCTS PROCESS DEVELOPMENT UNIT

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Genomic Analysis of Aspergillus Section Terrei Reveals a High Potential in Secondary Metabolite Production and Plant Biomass Degradation

Background/Objective

Species from *Aspergillus* section *Terrei* are known for producing secondary metabolites and degrading plant biomass. As such, this section is of significant interest due to its diversity of enzymes with application for biofuel and bioproduct production.

Approach

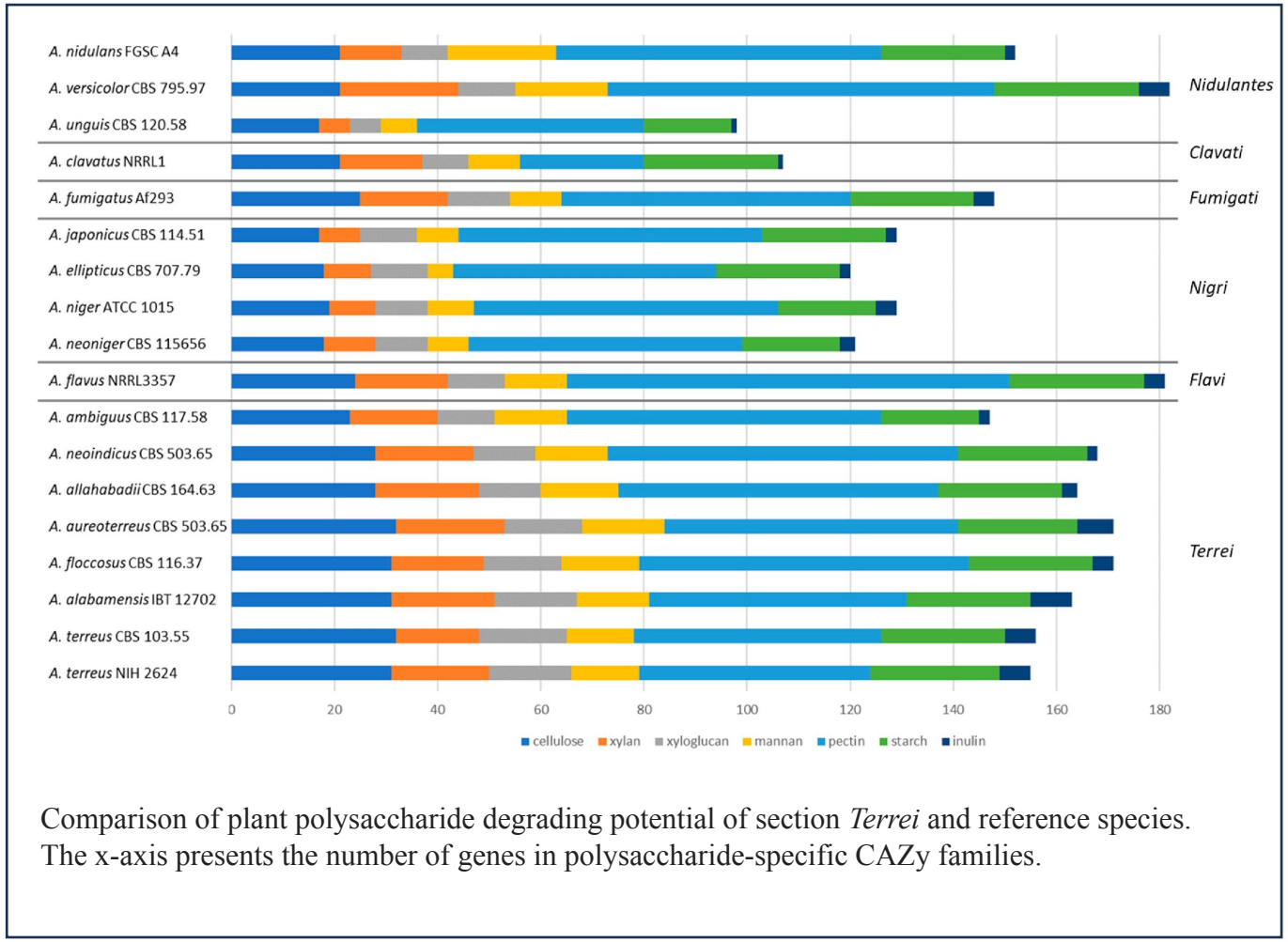
In collaboration with the JGI we sequenced, assembled, and annotated genomes from multiple *Aspergillus* section *Terrei* species to perform comparative genomic and phylogenetic analyses

Results

Our comparative genomic analyses of *Aspergillus* section *Terrei* significantly expanded the catalog of biotechnologically relevant genes involved in secondary metabolite production and plant biomass degradation

Significance/Impacts

The genomic analysis of *Aspergillus* section *Terrei* reveals a significant potential for secondary metabolite production and plant biomass degradation, presenting valuable prospects for industrial biotechnological applications



Comparison of plant polysaccharide degrading potential of section *Terrei* and reference species. The x-axis presents the number of genes in polysaccharide-specific CAZy families.

Theobald S., et. al. Journal of Fungi. doi: 10.3390/jof10070507

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Structural and biochemical basis for regiospecificity of the flavonoid glycosyltransferase UGT95A1

Background/Objective

Glycosylation is a predominant strategy plants employ to fine-tune the properties of small molecule metabolites to affect their bioactivity, transport, and storage.

Approach

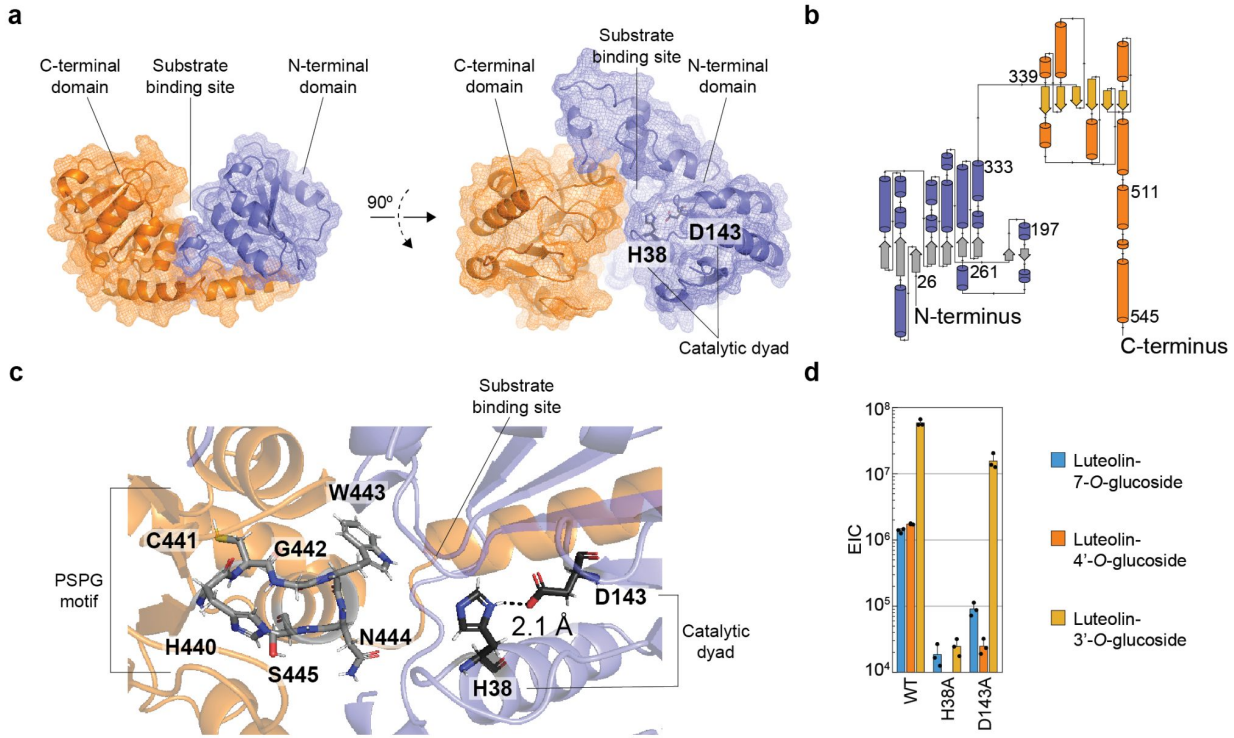
Here, we report a structural and biochemical investigation of UGT95A1 a family 1 GT enzyme from Pilosella officinarum that exhibits a strong, unusual regiospecificity for the 3'-O position of flavonoid acceptor substrate luteolin.

Results

Our study also suggests that the enzyme contains large, highly dynamic disordered regions that are important to both the overall efficiency and regiospecificity of the enzyme.

Significance/Impacts

This report represents a comprehensive in-depth analysis of a family 1 G enzyme with a unique substrate regiospecificity and may provide a basis for enzyme functional prediction and engineering.



Sirirungruang S., et. al. The Journal of biological chemistry. doi: 10.1016/j.jbc.2024.107602

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Verazine Biosynthesis from Simple Sugars in Engineered Saccharomyces cerevisiae

Background/Objective

- Steroidal alkaloids like cyclopamine and verazine (cyclopamine precursor) are high value compounds
- The microbial production of steroidal alkaloids could solve challenges associated with their natural or chemical production

Approach

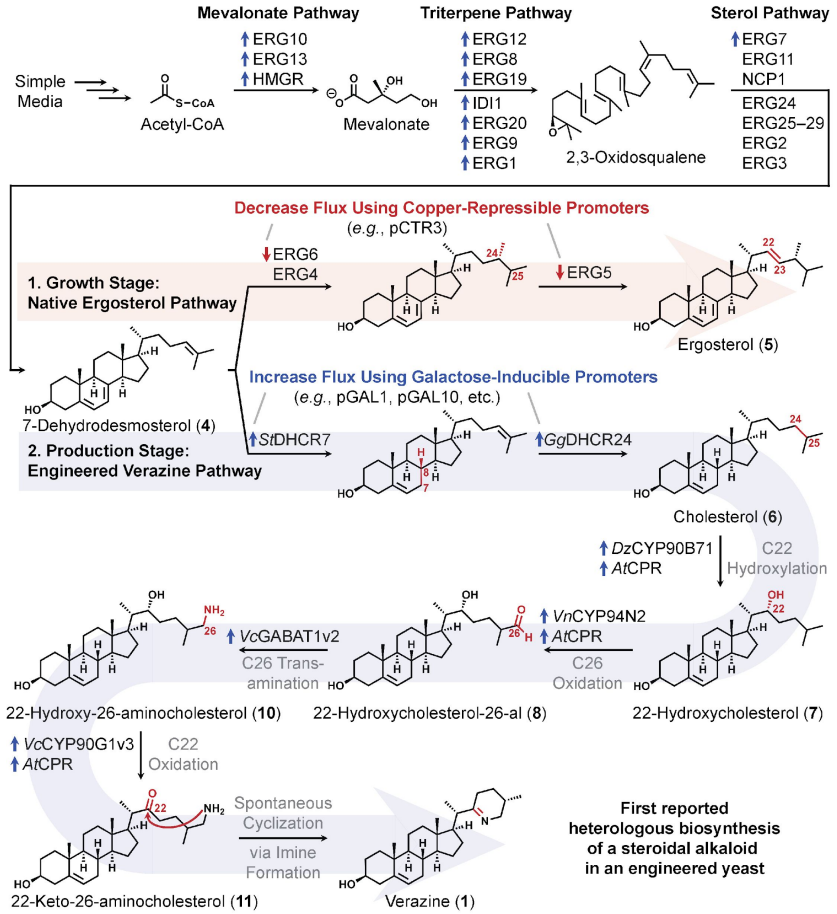
We engineered *S. cerevisiae* achieve an inducible switch from its native sterol (ergosterol) to cholesterol and introduced a refactored verazine biosynthetic pathway (8 enzymes from 7 different species).

Results

Verazine was biosynthesized in *S. cerevisiae* at a titer of 83 ± 3 µg/L and microbial-produced verazine was indistinguishable from natural or *Nicotiana benthamiana*-produced verazine

Significance/Impacts

This research lays the groundwork for future microbial biosynthesis of cyclopamine, (bio)synthetic derivatives of cyclopamine, and other steroidal alkaloid natural products



An inducible shift from ergosterol to cholesterol biosynthesis enabled a two-stage production strategy with separate growth and production stages (top). Verazine was biosynthesized from cholesterol through inducible expression of an engineered biosynthetic pathway (bottom).

Winegar P. H., et. al. Metabolic engineering. doi: 10.1016/j.ymben.2024.07.011

ENABLED PUBLICATIONS

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Guide RNA structure design enables combinatorial CRISPRa programs for biosynthetic profiling

Background/Objective

- CRISPR-Cas transcriptional control systems are emerging as important tools for programming multi-gene expression,
- But poor predictability of guide RNA folding can disrupt expression control.

Approach

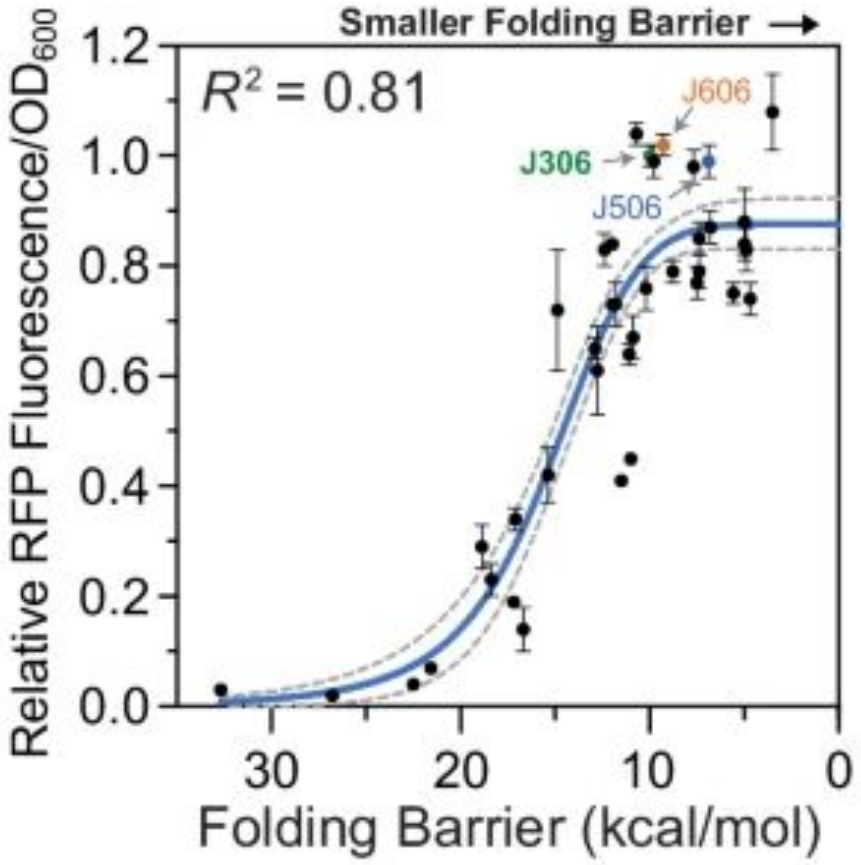
Here, we correlate efficacy of modified guide RNAs for CRISPR activation in *E. coli* with a computational kinetic parameter describing scRNA folding rate into the active structure.

Results

This parameter enables forward design of modified guide RNAs, allowing us to design a system of three synthetic CRISPRa promoters that can orthogonally activate (>35-fold) expression of chosen outputs.

Significance/Impacts

This RNA design approach may accelerate routine design of effective multi-gene regulation programs in bacterial hosts



Folding Barrier predicts the CRISPR-activated expression of promoter-scRNA pairs based on sequence.

Fontana, J., et. al. Nature communications. doi: 10.1038/s41467-024-50528-1