Programmable control of acetate metabolism in *Escherichia coli*

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Abstract

We are creating a genetic circuit that detects and responds to acetate production, a common problem in industrial fermentations of E. coli

This circuit integrates three metabolite signals, computes the cause of acetate production, and produces outputs that counteract these conditions

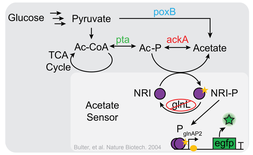
Genetic control enables individual cells to respond to the stresses they experience during industrial fermentation, which can improve growth and product yields

Sensing and reducing native acetate production

Δ*pta* Δ*poxB* eliminates acetate production, but slows growth rate

We will try to use feedback to regulate *pta* and *poxB* to eliminate acetate production w/o slowing growth

NRI acts as a strong acetate sensor in Δ*glnL* strains

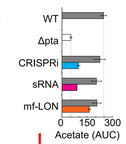


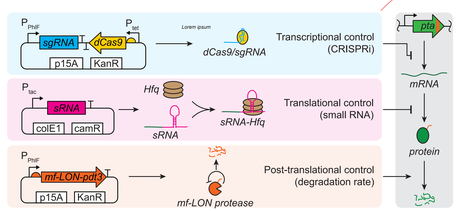
Controlling transcription, translation, and degradation of *pta* reduces acetate production

Developed inducible repression of acetate production genes
- CRISPRi[3], small RNA's, and *Mesoplasma florum* LON protease[4] act against native *pta* gene expression

Each approach downregulated target genes, but displayed different dynamics

We chose to use the mf-LON output due to its rapid degradation of the target gene.

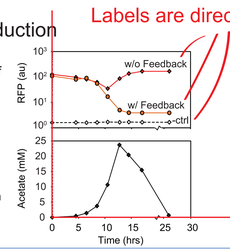




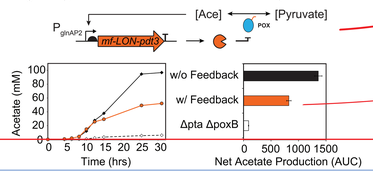
Direct feedback reduces acetate production

We used the acetate sensor (P_{glnAP2}) to drive expression of the mf-LON protease.

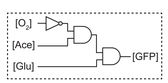
We observed strong, real-time control of RFP abundance in response to an endogenous increase in acetate production.



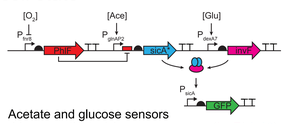
When mf-LON was targeted at the poxB enzyme (activated late in growth), acetate accumulation was reduced by 40%.



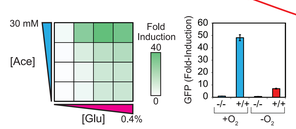
A digital circuit responds to metabolic cell state



We built a genetic program that integrates signals for acetate, glucose, and oxygen which
- Activates GFP production only when acetate is made in response to glucose
- Deactivates GFP production in microaerobic conditions



Acetate and glucose sensors drive an activator/chaperone system (invF/sicA*), forming an AND gate. A native oxygen sensor drives production of a PhlF repressor that downregulates the acetate-sensitive promoter.



The system is sensitive to low glucose levels and shows up to 20-fold activation 7.5 mM acetate.

Liberal use of whitespae around poster border, poster title, authors, logos, and section titles draws attention and maximizes readability

The line break in the title is at a meaningful place, not just where both lines would be the same length or using the entire width of the poster

Each thought is physically separated by line breaks to avoid the "block of text," which can be difficult to approach

Each point is descriptive, but concise

The abstract includes the impact and the scope of the work. It allows people passing by to quickly assess whether or not to engage with the poster

This section provides biological context, which can help the audience to arrive at their own conclusions about the validity and scope of the work

Concept is portrayed visually rather than with text, making it easier to provide a lot of information that is easy to understand

Highlighting boxes show that concepts are grouped horizontally rather than vertically. Not using a border on the boxes makes them continuous with the rest of the section, rather than being blocked off

Consistent color scheme demonstrates continuity of concepts. Plot and boxes are in the same order as well, which is also the order that the methods act in biologically

The title of the section provides the main takeaway

Graphics and text are are synergistically redundant (they both demonstrate that the feedback acts by targeting poxB)

Results are stated in plain English and are placed next to the plots that they refer to

Color is used to draw attention to the condition that portrays the main message of the section

Plots' axes are aligned to create a clean look

Plots that have a synergistic message are grouped together. The left group of plots share an x-axis to demonstrate two different pieces of data gathered on the same time course

Using a small amount of color in the text enables quick reference to the concepts they refer to in the schematic