Burdensome gene expression promotes heterogeneity in cellular response that can be mitigated up to a tipping point

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Introduction

The activation of burdensome gene circuits is marked by significant cell-to-cell heterogeneity in induction levels. Whether applied in bioprocessing, cellular ecology, or biomedical contexts, there is considerable interest into comprehending and mitigating this heterogeneity.

Findings

This study focuses on the *E. coli* BL21 T7 expression system as a representative example of a burdensome gene circuit. Utilizing a quantitative measure of heterogeneity, entropy, we observed that periodic pulsing of the inducer could homogenize the cell-to-cell expression levels of the T7 expression system in the context of a continuous cultivation. We examined the population's rebound to a heterogeneous structure and proposed that this heterogeneity serves as a survival strategy, allowing the population to evade washout from its environment. Indeed, harsh induction drastically reduce the growth rate of cells and thus we propose that the population avoids washout via a mechanism that we define as 'entropy compensation' where a fraction of cell that did not activate the gene circuit rapidly takes over. To explore the limits of this survival strategy, we increased the pulsing frequency, aiming to push the overall induction level to a point where washout becomes unavoidable. Intriguingly, despite demonstrating the system's tunability with respect to pulse frequency, no washout occurred. Instead, a new sub-population with a lower induction level emerged and gradually dominated. Subsequent sequencing revealed that this population harbored a mutation weakening the expression strength. In summary, our work highlights the instability of the BL21 T7 expression system, showcases its ability to adapt through heterogeneity as a survival strategy and reveals a genetic escape when trying to mitigate phenotypic heterogeneity and push up induction.

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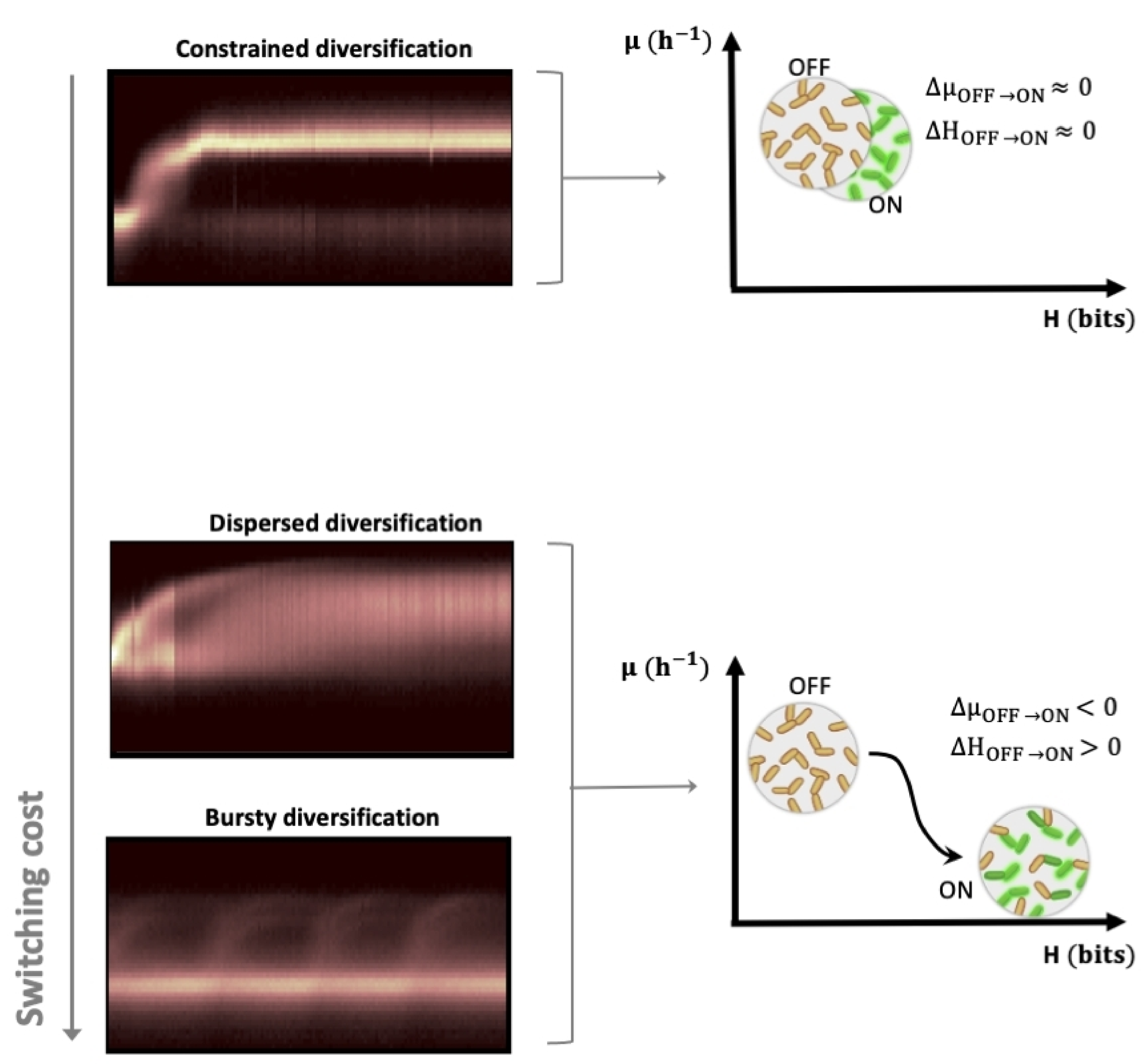
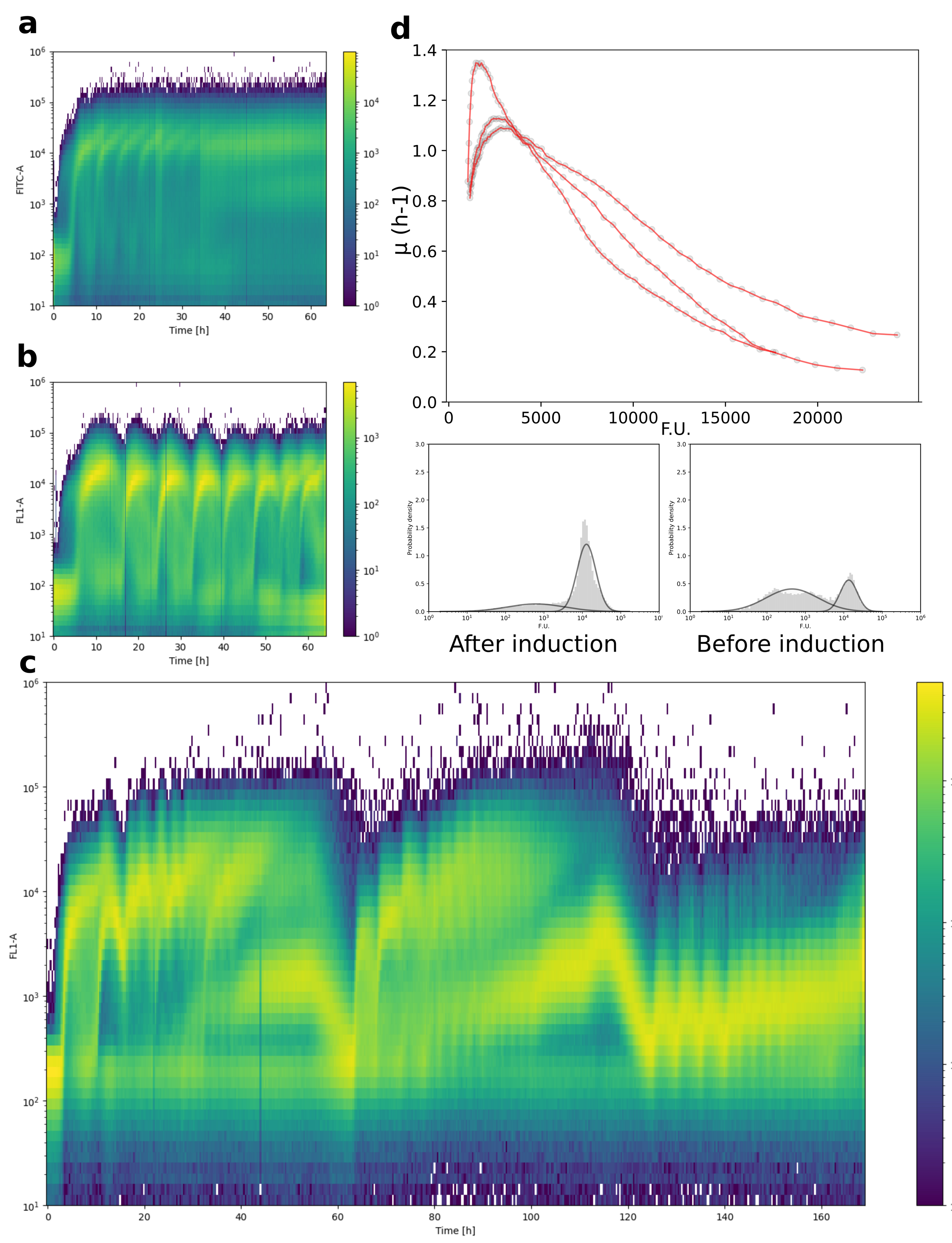
Background



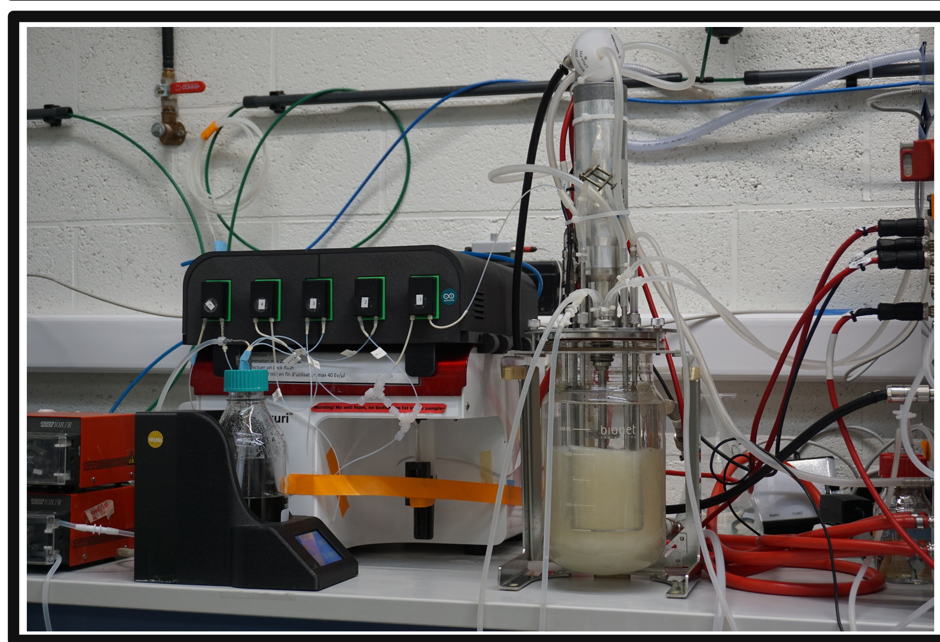
We have previously observed that as the fitness cost associated with the activation of a gene circuit increases, the population becomes more and more heterogeneous. Based on this, we identified three diversification regimes: 1) constrained for low switching cost; 2) dispersed for intermediate cost; 3) bursty, where the population synchronizes itself, for high fitness cost, such as sporulation in *Bacillus subtilis*.

Henrion, L., Martinez, J.A., Vandenbroucke, V. et al. Fitness cost associated with cell phenotypic switching drives population diversification dynamics and controllability. Nat Commun 14, 6128 (2023). https://doi.org/10.1038/s41467-023-41917-z

Results



a) Chemostat cultivations of *E. coli* BL21 are characterized by a significant degree of heterogeneity in induction (up to 10,000x) of the T7 expression system.
b) When applying a pulse-based strategy, the population is globally more homogeneous, and diversification occurs during the de-induction phase.
d) Analysis of the growth rate per fluorescence level reveals a "growth entropy compensation" mechanism where cells that were not induced overgrow the others, thus amplifying population heterogeneity.
c) Trying to circumvent this phenomenon by pulsing faster and faster leads to the occurrence of a sub-population composed of a mutant of BL21 with a weakened lac promoter.



The Segregostat

In our lab, we have developed a new cell-machine interface that draws a sample from a bioreactor every 12 minutes and analyzes 20,000 cells. Based on this analysis and predefined control rules, peristaltic pumps can be activated to exert control over the microbial population.