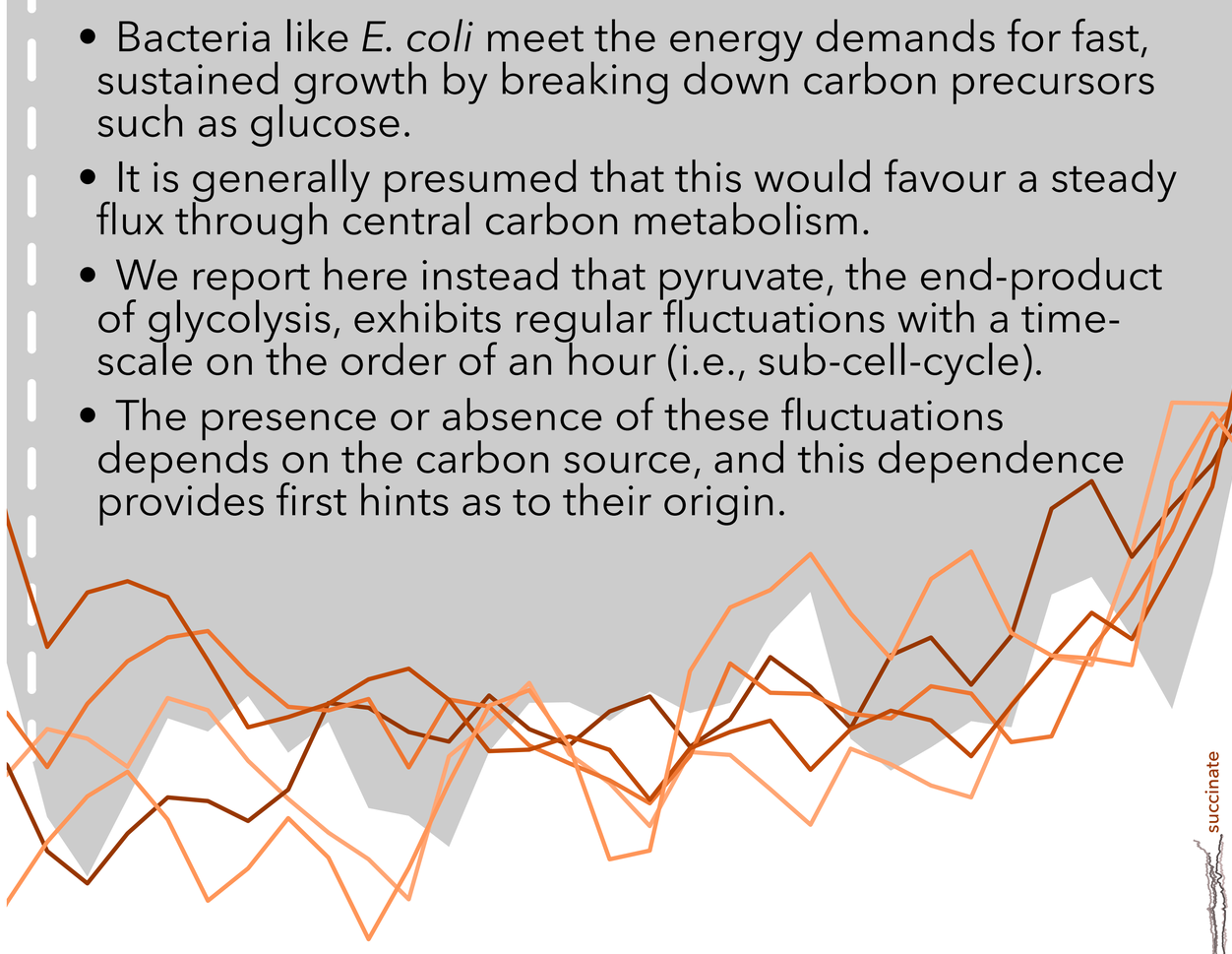
Regular fluctuations in central carbon metabolism are carbon-source dependent in *E. coli*

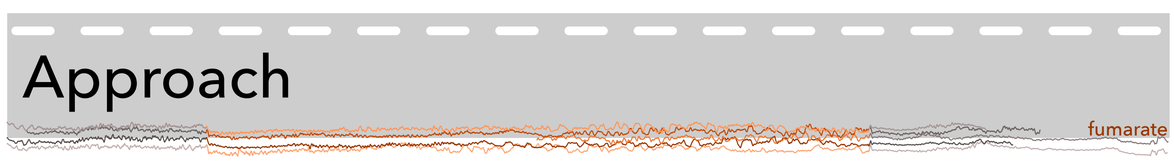
Julian Pietsch and Victor Sourjik

MAX PLANCK INSTITUTE
FOR TERRESTRIAL MICROBIOLOGY

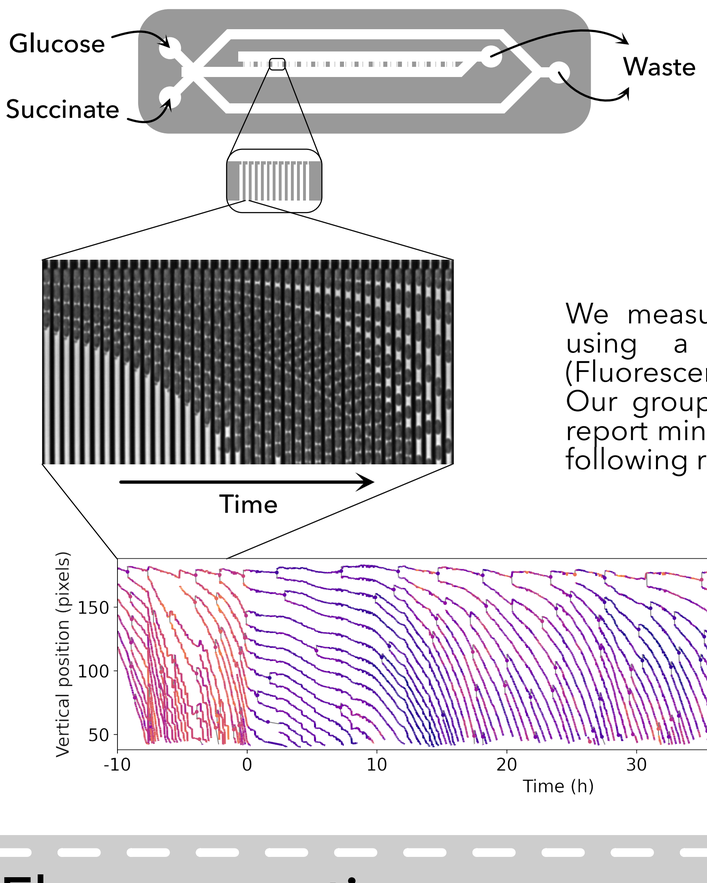
- Bacteria like *E. coli* meet the energy demands for fast, sustained growth by breaking down carbon precursors such as glucose.
- It is generally presumed that this would favour a steady flux through central carbon metabolism.
- We report here instead that pyruvate, the end-product of glycolysis, exhibits regular fluctuations with a time-scale on the order of an hour (i.e., sub-cell-cycle).
- The presence or absence of these fluctuations depends on the carbon source, and this dependence provides first hints as to their origin.

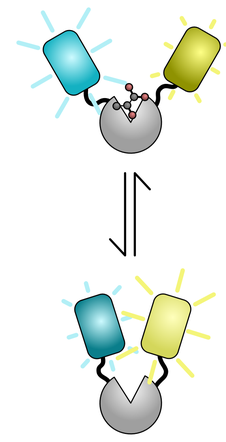


Approach



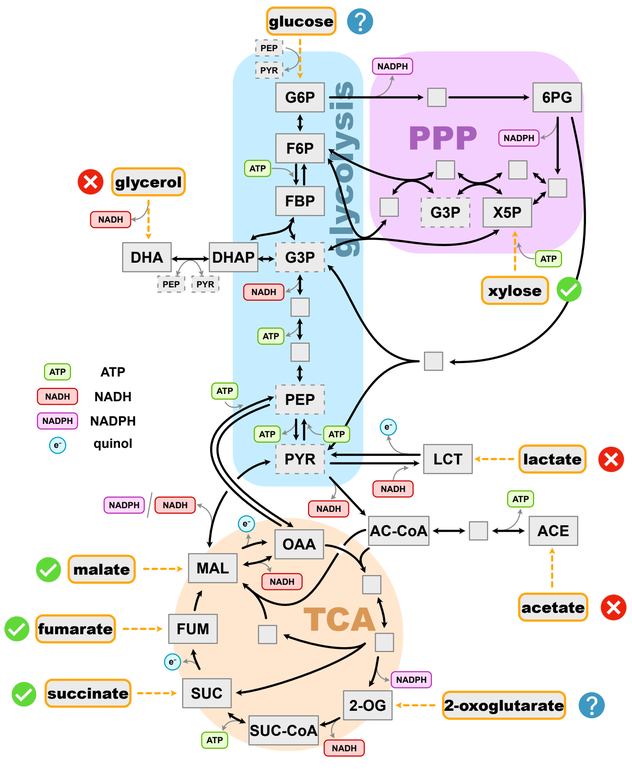
We follow growth of cells across many generations using a **mother machine** device with microfluidic control to switch between alternative carbon sources.





We measure pyruvate levels in live cells using a biosensor based on **FRET** (Fluorescence Resonance Energy Transfer). Our group previously used this sensor to report minute-scale fluctuations in pyruvate following rapid changes in metabolic flux[1].

Flux accounting



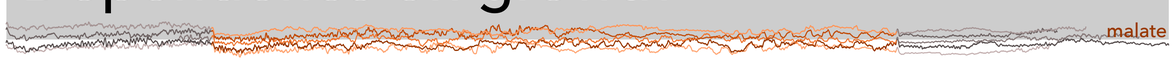
TL;DR

- Here we report that during steady-state growth on certain carbon sources, pyruvate exhibits fluctuations on a time scale shorter than the cell-division cycle.
- These fluctuations resemble those previously observed for ATP[2], but their carbon-source dependence is different.
- Pyruvate fluctuations are strongest for growth on late TCA cycle intermediates, but are also seen for growth on a glycolytic carbon source (xylose).
- Notably, fluctuations are not observed for glycerol, which feeds into lower glycolysis, or for lactate, which makes use of an alternative respiratory chain.
- Flux consideration indicates a potential role for redox balance in relaying fluctuations to pyruvate.

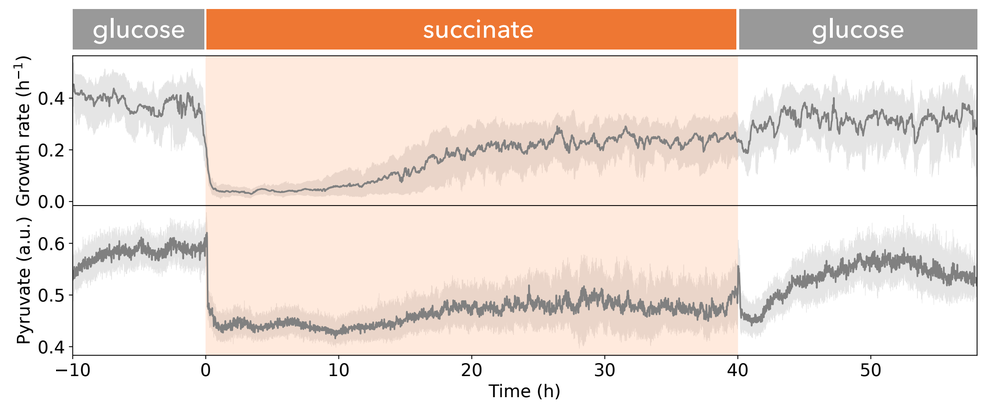
succinate

2-oxoglutarate

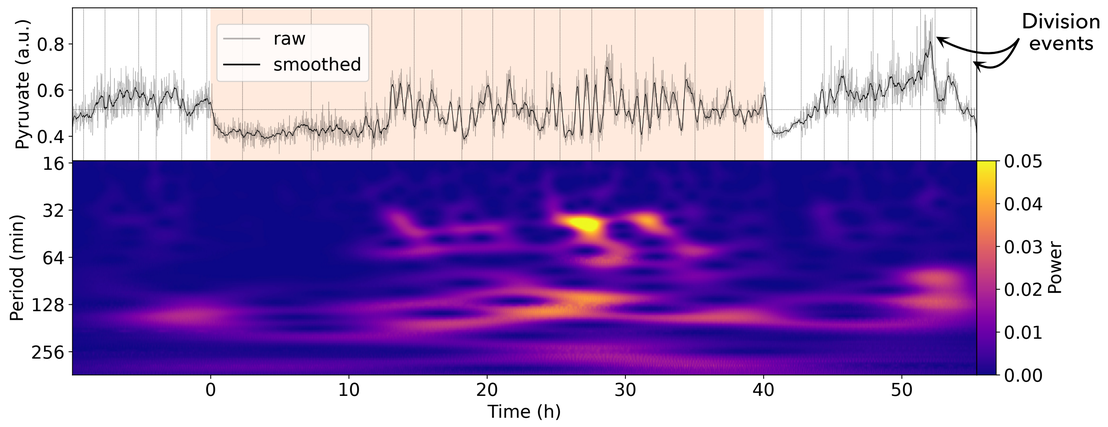
Dependence on growth



At the **population level**, growth rate and pyruvate levels drop upon a sudden shift from a glycolytic to a gluconeogenic carbon source, and only slowly recover to steady-state.

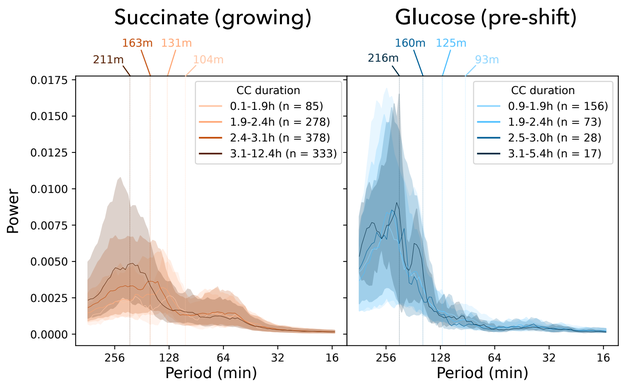


In **single cells**, pyruvate levels start fluctuating once cells resume growth, with a period on the order of an hour. The hour-scale fluctuations stop when shifting back to glucose.



By averaging the wavelet scalogram over time, we can obtain spectral estimates for each cell cycle.

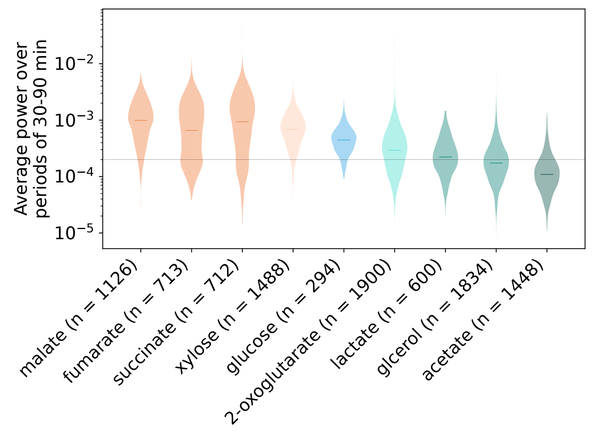
This reveals a dependence of the division-scale fluctuations on cell-cycle duration, but not the hour-scale fluctuations.



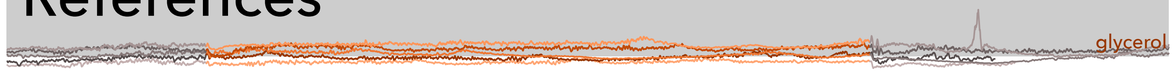
Dependence on carbon-source

The above experiment and analysis was repeated for selected carbon sources, chosen to target different entry-points into central carbon metabolism.

Focussing on the power for periods in the range of 30 to 90 minutes, we observe large variations in the distribution over cell cycles as a function of carbon source, including bimodality.



References



1. Bi, S., Kargeti, M., Colin, R., Farke, N., Link, H., and Sourjik, V. (2023). Dynamic fluctuations in a bacterial metabolic network. *Nat Commun*, 14(1):2173.
2. Lin, W.-H. and Jacobs-Wagner, C. (2022). Connecting single-cell ATP dynamics to overflow metabolism, cell growth, and the cell cycle in *Escherichia coli*. *Curr Biol*, 32(18):3911–3924.e4.