

Supplemental material for: Fundamental limits on the rate of bacterial cell division

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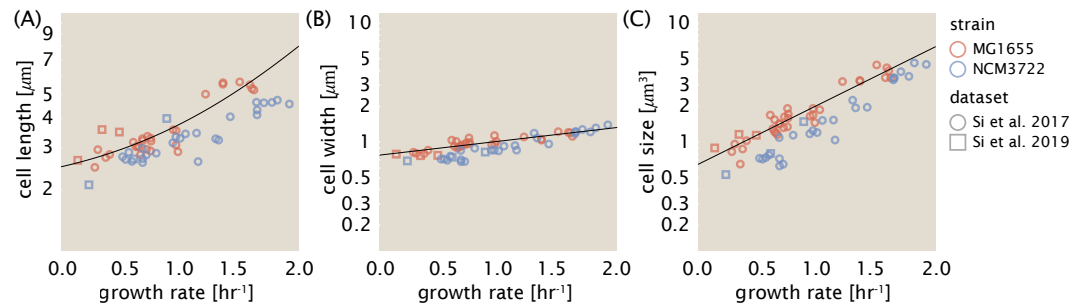


Figure 1. Summary of size measurements from Si *et al.* 2017, 2019. Cell lengths and widths were measured from cell contours obtained from phase contrast images, and refer to the long and short axis respectively. (A) Cell lengths and (B) cell widths show the mean measurements reported (they report 140-300 images and 5,000-30,000 for each set of samples; which likely means about 1,000-5,000 measurements per mean value reported here since they considered about 6 conditions at a time). Fits were made to the MG1655 strain data; length: $0.5 e^{1.09 \cdot \lambda} + 1.76 \mu\text{m}$, width: $0.64 e^{0.24 \cdot \lambda} \mu\text{m}$. (C) Cell size, V , was calculated as cylinders with two hemispherical ends (Equation 1). The MG1655 strain data gave a best fit of $0.533 e^{1.037 \cdot \lambda} \mu\text{m}^3$.

Estimation of cell size and surface area across all growth conditions.

In Figure ?? we looked at a number of recent cell size measurements and potential issues with the values used by Schmidt *et al.*. Since most of the proteomic data sets lack cell size measurements, we chose instead to use a common set of size measurements for any analysis requiring cell size or surface area. Since each of the data sets used either K-12 MG1655 or its derivative, BW25113 (from the lab of Barry L. Wanner; the parent strain of the Keio collection (Datsenko and Wanner, 2000; Baba *et al.*, 2006)), we fit the MG1655 cell size data from Si *et al.* 2017, 2019 using the `optimize.curve_fit` function from the `Scipy` python package (Virtanen *et al.*, 2020).

The size data is shown in Figure 1)(A) and (B), for the cell length and width, respectively. The length data was well described by the exponential function $0.5 e^{1.09 \cdot \lambda} + 1.76 \mu\text{m}$, while the width data was well described by $0.64 e^{0.24 \cdot \lambda} \mu\text{m}$. In order to estimate cell size we take the cell as a cylinders with two hemispherical ends (Si *et al.*, 2017; Basan *et al.*, 2015). Specifically, cell size (or volume) is estimated from,

$$V = \pi \cdot r^2 \cdot (l - 2r/3), \quad (1)$$

where r is half the cell width. A best fit to the data is described by $0.533 e^{1.037 \cdot \lambda} \mu\text{m}^3$. Calculation of the cell surface area is given by,

$$S = \eta \cdot \pi \left(\frac{\eta \cdot \pi}{4} - \frac{\pi}{12} \right)^{-2/3} V^{2/3}, \quad (2)$$

where η is the aspect ratio ($\eta = l/w$) (Ojkic *et al.*, 2019).

Extending Estimates to a Continuum of Growth Rates

In the main text, we considered a standard stopwatch of 5000 s to estimate the abundance of the various protein complexes considered. In addition to point estimates, we also showed the estimate as a function of growth rate as transparent grey curves. In this section, we elaborate on this continuum estimate and compare and contrast the approach to the point estimate procedure.

Estimation of the total cell mass

For many of the processes estimated in the main text we relied on a cellular dry mass of $\approx 300 \text{ fg}$ from which we computed elemental and protein fractions using knowledge of fractional composition of the dry mass. At modest growth rates, such as the 5000 s doubling time used in the main text, this is a reasonable number to use as the typical cell mass is $\approx 1 \text{ pg}$ and *E. coli* cells can approximated as 70% water by volume. However, as we have shown in this supplemental information, the cell

size and therefore cell volume is highly dependent on the growth rate. This means that a dry mass of 300 fg cannot be used reliably across all growth rates.

Rather, using

References

- Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K. A., Tomita, M., Wanner, B. L., and Mori, H. (2006). Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Molecular Systems Biology*, 2(1):2460.
- Basan, M., Zhu, M., Dai, X., Warren, M., Sévin, D., Wang, Y.-P., and Hwa, T. (2015). Inflating bacterial cells by increased protein synthesis. *Molecular Systems Biology*, 11(10):836.
- Datsenko, K. A. and Wanner, B. L. (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences*, 97(12):6640–6645.
- Ojkic, N., Serbanescu, D., and Banerjee, S. (2019). Surface-to-volume scaling and aspect ratio preservation in rod-shaped bacteria. *eLife*, 8:642.
- Si, F., Li, D., Cox, S. E., Sauls, J. T., Azizi, O., Sou, C., Schwartz, A. B., Erickstad, M. J., Jun, Y., Li, X., and Jun, S. (2017). Invariance of Initiation Mass and Predictability of Cell Size in *Escherichia coli*. *Current Biology*, 27(9):1278–1287.
- Virtanen, P., Gommers, R., Oliphant, T. E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S. J., Brett, M., Wilson, J., Jarrod Millman, K., Mayorov, N., Nelson, A. R. J., Jones, E., Kern, R., Larson, E., Carey, C., Polat, İ., Feng, Y., Moore, E. W., Vand erPlas, J., Laxalde, D., Perktold, J., Cimrman, R., Henriksen, I., Quintero, E. A., Harris, C. R., Archibald, A. M., Ribeiro, A. H., Pedregosa, F., van Mulbregt, P., and Contributors, S. . . (2020). SciPy 1.0: Fundamental Algorithms for Scientific Computing in Python. *Nature Methods*, 17:261–272.