Synthesis and degradation of FtsZ determines the first cell division in starved bacteria

Supplementary Information

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1 Development and parametrization of the FtsZ model

1.1 Introduction

This section discusses how the FtsZ model was developed and parameterized with values from previous literature. We started with the most basic model possible for the FtsZ threshold activation of division and used a differential equation to describe how FtsZ abundance changes. Starting with the equation from [10], we considered just the synthesis and degradation of FtsZ toward its changing abundance. We assumed dilution effects to be neglible because the cells are not dividing. Furthermore, we expect the number of FtsZ per cell to dictate division occurrence, not the intracellular concentration. For synthesis, we assumed that the yield of FtsZ on carbon is constant and, therefore, can be approximated as a TI feedrate dependent synthesis (αf):

$$\frac{d[\mathrm{FtsZ}]}{dt} = \alpha f - \frac{V_{\max}[\mathrm{FtsZ}]}{K_m + [\mathrm{FtsZ}]} \tag{1}$$

Considering the abundance of FtsZ throughout stationary phase [8], we found that the level remains roughly constant (700 copies/cell) between 1 day and 3 days in stationary phase. This suggests that FtsZ never fully depletes during stationary phase, and that our model was oversimplified. To account for this, we posed an additional term for the basal synthesis of FtsZ (α_0):

$$\frac{d[\text{FtsZ}]}{dt} = \alpha_0 + \alpha_1 f - \frac{V_{\max}[\text{FtsZ}]}{K_m + [\text{FtsZ}]} \tag{2}$$

1.2 Closed Form Solution for the Michaelis-Menten Equation

A closed form solution to time-dependent enzyme kinetics, based on the Michaelis-Menten rate law, was first described in 1997 by Schnell and Mendoza [9]. Using the quasi-steady-state approximation, the differential equation that describes the concentration of a substrate S that is degraded by and enzyme with a maximum velocity of $V_{\max}$ is given by:

$$\frac{d[\text{S}]}{dt} = -\frac{V_{\max}[\text{S}]}{K_M + [\text{S}]} \tag{3}$$

Solving this equation for $[S](t)$ yields:

$$[\text{S}](t) = K_M \cdot W\left[\frac{[\text{S}]_0}{K_M} \exp\left(\frac{[\text{S}]_0 - V_{\max}\ t}{K_M}\right)\right] \tag{4}$$

Where $[\text{S}]_0$ is the value of [S] at time $t = 0$, and $W[\cdot]$ is the *Lambert-W* function (also known as the *omega* function or the *product logarithm*) and is defined as the inverse of the function $z \to z \cdot e^z$ (see [3]).

In our work, however, this solution is not sufficient, since we are dealing with constant positive production on top of the enzymatic degradation, i.e. $\alpha_0 + \alpha_1 f$. Therefore, we modified equation 3 to be:

$$\frac{d[\text{S}]}{dt} = v_{\text{in}} - \frac{V_{\max}[\text{S}]}{K_M + [\text{S}]} \tag{5}$$

The solution in this more general case is:

$$[\text{S}](t) = [\text{S}]_\infty + (K_M + [\text{S}]_\infty) \cdot W\left[\frac{[\text{S}]_0 - [\text{S}]_\infty}{K_M + [\text{S}]_\infty}\ \exp\left(\frac{[\text{S}]_0 - [\text{S}]_\infty - (V_{\max} - v_{\text{in}})t}{K_M + [\text{S}]_\infty}\right)\right] \tag{6}$$

where we define $[\text{S}]_\infty \equiv K_M \frac{v_{\text{in}}}{V_{\max} - v_{\text{in}}}$, i.e. the value of [S] at steady-state (or $t \to \infty$).

Note that if $v_{\text{in}} > V_{\max}$, the value of $[\text{S}]_\infty$ will be negative, indicating that the system has no steady-state (and [S] continues to grow indefinitely). Nevertheless, equation 6 holds even in such cases, except that we need to use the lower branch of the Lambert-W function (usually denoted W_{-1}).

It is sometimes useful to consider the inverse function of $[\text{S}](t)$, as we will soon see for predicting the lag time using [FtsZ] levels. Note that since the differential equation

(5) is time invariant, there is a unique solution for the time difference $\Delta t = t_1 - t_0$, given the initial and final concentrations $[\mathrm{S}](t_0)$ and $[\mathrm{S}](t_1)$. The solution is given by:

$$\Delta t = \frac{1}{v_{\mathrm{in}} - V_{\max}} \cdot \left([\mathrm{S}](t_1) - [\mathrm{S}](t_0) - (K_M + [\mathrm{S}]_\infty) \ln \left(\frac{[\mathrm{S}](t_1) - [\mathrm{S}]_\infty}{[\mathrm{S}](t_0) - [\mathrm{S}]_\infty} \right) \right) \tag{7}$$

1.3 Applying the Closed Form Solution

In the case of [FtsZ], we could now apply equation 6 which will be of the following form:

$$[\mathrm{FtsZ}](t) = [\mathrm{FtsZ}]_\infty + (K_M + [\mathrm{FtsZ}]_\infty) \cdot W_i \left(a\, e^{a - b\, t} \right) . \tag{8}$$

where

$$\begin{aligned} [\mathrm{FtsZ}]_\infty &\equiv K_M \frac{\alpha_0 + \alpha_1 f}{V_{\max} - \alpha_0 - \alpha_1 f} \\ a &\equiv \frac{[\mathrm{FtsZ}]_0 - [\mathrm{FtsZ}]_\infty}{K_M + [\mathrm{FtsZ}]_\infty} \\ b &\equiv \frac{V_{\max} - \alpha_0 - \alpha_1 f}{K_M + [\mathrm{FtsZ}]_\infty} \end{aligned} \tag{9}$$

and $W_i = W_0$ in case $V_{\max} > \alpha_0 + \alpha_1 f$ and $W_i = W_{-1}$ otherwise.

In order to answer the question what is the time t at which [FtsZ] accumulate from an initial concentration (F_I) to the threshold concentration required for division (F_T), we could use the inverse formula, i.e. equation 7:

$$t_{\mathrm{lag}} = \frac{1}{\alpha_0 + \alpha_1 f - V_{\max}} \cdot \left(F_T - F_I - (K_M + [\mathrm{FtsZ}]_\infty) \ln \left(1 + \frac{F_T - F_I}{F_I - [\mathrm{FtsZ}]_\infty} \right) \right) . \tag{10}$$

As one would expect, this function diverges ($t_{\mathrm{lag}} \to \infty$) when $[\mathrm{FtsZ}]_\infty \to F_T$. Since $[\mathrm{FtsZ}]_\infty$ is a function of the TI feedrate, we could solve for f from equation 2 and find that this happens when

$$f_{\mathrm{critical}} = \frac{1}{\alpha_1} \left(V_{\max} \frac{F_T}{K_M + F_T} - \alpha_0 \right) .$$

For any $f < f_{\mathrm{critical}}$, the production rate of FtsZ is too low and its level would never reach F_T.

Before using this closed form solution to predict lag times, we had to first find the values for the system parameters, namely α_0, α_1, K_M, $V_{\max}$, F_I, and F_T.

1.4 Parametrization

We first parametrized the degradation term using *in vitro* measurements from previous work [2]. We adapted data from Figure 1F within [2] to find the turnover number of FtsZ into the ClpXP protease complex. The data shows the degradation of FtsZ over 90

minutes depending on the concentration of ClpX in 25 μL total volume. We considered two points seemingly within the linear range of degradation:

ClpXP concentration [μM]	pmol FtsZ degraded/90 min	turnover [min^{-1}]
0.05	5	0.044
0.30	40	0.059

Roughly, we have a turnover number of 0.05 min^{-1}. For the actual $V_{\max}$, we need to consider the number of active ClpXP within *E. coli*. From [4], we know that ClpX is stochiometrically limiting toward formation of the ClpXP complex. Therefore, to calculate $V_{\max}$, we can simply consider the number of ClpX in *E. coli*. In starved conditions, there is approximately 200 copies of ClpX per cell [8]. Our $V_{\max}$ is approximated to be $200 \cdot 0.05 = 10$ [cell^{-1}min^{-1}].

Unfortunately, K_M is difficult to resolve using data from [2]. We, therefore, just used the same value from an earlier, non-specific protein degradation kinetics study [10] where $K_M = 600$ [cell^{-1}].

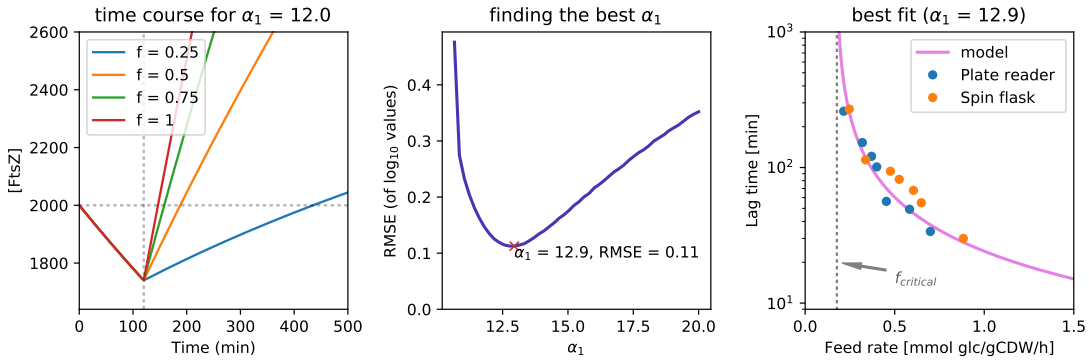
We could now use the final steady-state condition to calculate the basal synthesis term (α_0). Per the observation that FtsZ maintains a steady-state abundance of 700 copies per cell in stationary phase, which means that $[\text{FtsZ}]_\infty = 700$ [cell^{-1}], when $f = 0$.

$$\begin{aligned} [\text{FtsZ}]_\infty &= K_M \frac{\alpha_0 + \alpha_1 \cdot 0}{V_{\max} - \alpha_0 - \alpha_1 \cdot 0} \\ \alpha_0 &= V_{\max} \frac{[\text{FtsZ}]_\infty}{K_M + [\text{FtsZ}]_\infty} = 5.4 \text{ [cell}^{-1}\text{min}^{-1}\text{]} \end{aligned} \tag{11}$$

We then had enough information to predict how FtsZ depletes during starvation without feeding. Per [8], we see that number of FtsZ under growing conditions is about 2000 copies/cell. We took this value to be the FtsZ concentration both at the onset of starvation as well as the threshold F_T needed to induce cell division. Using the above parameters from literature and plugging them into equation (6), we calculated that FtsZ levels after 2 hours of starvation would deplete to 1740 copies/cell. This value was taken to be the starting FtsZ at the onset of pulsing, i.e. $F_I = 1740$ [cell^{-1}]. The values for all the model parameters are summarized in Supplementary Table 6.

1.5 Fitting α_1

Now, the only missing parameter was α_1, i.e. the yield of FtsZ from fed carbon (in units of $\left[\frac{\text{cell}^{-1}\text{min}^{-1}}{\text{mmol glc/g DCW/h}}\right]$). Since there is no available data for this relationship, we fitted the value of α_1 based on our TI feedrate versus lag time data and the results from equation (10), as illustrated in Supplementary Fig 12. Since the lag time spreads for lower feedrates, we applied a log transform on the lag times prior to fitting. We did not consider data point where no visible lag time was detected because our FtsZ model assumes $t_{\text{lag}} > 0$. We found that $\alpha_1 = 12.9 \left[\frac{\text{cell}^{-1}\text{min}^{-1}}{\text{mmol glc/g DCW/h}}\right]$.



Supplementary Figure 12: Fitting α_1. Given a set of parameters, our model simulates the value of [FtsZ] over time. We then fitted the value of α_1 to give the best estimate for the measured lag times (as a function of TI feedrate f). α_1 is given in units of $\left[\frac{\text{cell}^{-1}\text{min}^{-1}}{\text{mmol glc/g DCW/h}}\right]$.

We could now rewrite the lag time as a direct function of the TI feedrate f:

$$t_{\text{lag}}(f) = \frac{F_I - F_T}{V_{\max} - \alpha_0 - \alpha_1 f} - \frac{K_M V_{\max}}{(V_{\max} - \alpha_0 - \alpha_1 f)^2} \ln\left(1 + \frac{(F_T - F_I)(V_{\max} - \alpha_0 - \alpha_1 f)}{F_I(V_{\max} - \alpha_0 - \alpha_1 f) - K_M(\alpha_0 + \alpha_1 f)}\right)$$

and calculate the critical TI feedrate:

$$f_{\text{critical}} = \frac{1}{\alpha_1}\left(V_{\max}\frac{F_T}{K_M + F_T} - \alpha_0\right) = 0.16 \text{ mmol glc/g DCW/h}\,. \tag{12}$$

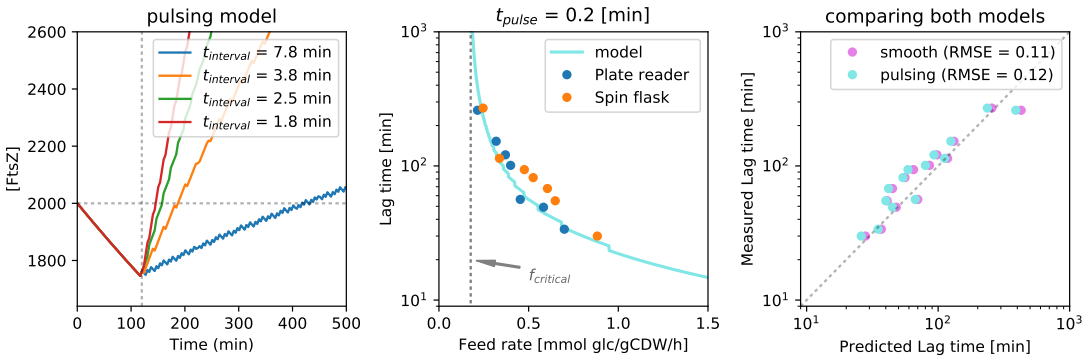
This is very close to our experimentally measured threshold of non-division (over the first 6 hours), which was at 0.2 mmol glc/g DCW/h.

Note that although $t_{\text{lag}}(f)$ seems to have a singularity point at $f = \frac{V_{\max} - \alpha_0}{\alpha_1}$ (in our case it is equal to 0.355 mmol glc/g DCW/h), the function is continuous finite at the entire range of $(f_{\text{critical}}\ ,\ \infty)$.

1.6 Taking the pulsing into account

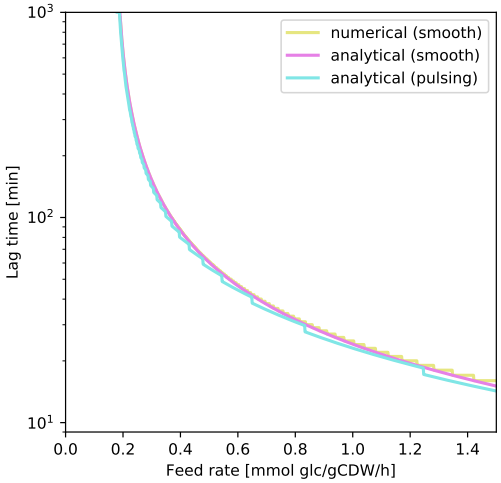
It is important to remember that our solution for the estimated lag time (equation 10) assumes that after the starvation phase, there is a steady production of [FtsZ] ($\alpha_0 + \alpha_1 f$). However, our experimental system provides a pulse of glucose every 2-10 minutes, and the cells consume that glucose within ≈ 0.2 minutes (assuming a maximal glucose uptake rate of 10 mmol glc/g DCW/h [6]).

In order to check whether the smooth simplification (denoted the *smooth* model) alters the predicted lag phases, we redid our calculations without this assumption, by precisely tracking the changes in FtsZ production rates during the pulse (i.e. while glucose is present) and between the pulses (i.e. while the glucose level is 0). The results are presented in Supplementary Figure 13, and show that the effect is negligible.



Supplementary Figure 13: Analytical solution for a pulsing input function.

Finally, we compared the lag time predictions of the *smooth* and *pulsing* models, assuming the same average TI feedrate. In addition, we solved the ODE system numerically by integrating over time and finding the point where [FtsZ] crosses the threshold F_T. We conclude that the differences between all three models are negligible (Supplementary Figure 14).



Supplementary Figure 14: All three models give very similar predictions for the lag time.

All code used for parametrization and model generation is available in Supplementary Material or at `https://github.com/karsekar/pulsefeeding-analysis`.

2 Supplementary Tables

Supplementary Table 4: Strains used in this study

Strain	Genotype	Description
BW 25113	Δ(araD-araB)567 Δ(rhaD-rhaB)568 ΔlacZ4787 (::rrnB-3) hsdR514 rph-1	Parent strain from Keio Collection [1]. Used as wild-type (WT).
Δ*crp*	Same as BW 25113 with Δcrp. Kanamycin marker was excised from corresponding strain from [1].	*crp* deletion strain.
Δ*pdhR*	Same as BW 25113 with ΔpdhR. Kanamycin marker was excised from corresponding strain from [1].	*pdhR* deletion strain.
Δ*clpX*	Same as BW 25113 with ΔclpX. Strain from Keio collection [1]. Kan marker was not removed.	*clpX* deletion strain.
Δ*clpP*	Same as BW 25113 with ΔclpP. Strain from Keio collection [1]. Kan marker was not removed.	*clpP* deletion strain.

Supplementary Table 5: Plasmids used in this study

Plasmid	Description	Reference
epd-icd	Constitutive GFP plasmid with SC101 backbone.	[5]
pJKR-L-tetO	Parent plasmid for titrated synthesis of GFP with tetracycline-based inducer. SC101 backbone.	[7]
pPLtetO-PdhR	Derived from pJKR-L-tetO. Titrates synthesis of PdhR with tetracycline-based inducer.	This study.
pPLtetO-FtsZ	Derived from pJKR-L-tetO. Titrates synthesis of FtsZ with tetracycline-based inducer.	This study.
pPLtetO-ClpX	Derived from pJKR-L-tetO. Titrates synthesis of ClpX with tetracycline-based inducer.	This study.
pPLtetO-FtsA	Derived from pJKR-L-tetO. Titrates synthesis of FtsA with tetracycline-based inducer.	This study.
pPLtetO-FtsB	Derived from pJKR-L-tetO. Titrates synthesis of FtsB with tetracycline-based inducer.	This study.
pPLtetO-FtsN	Derived from pJKR-L-tetO. Titrates synthesis of FtsN with tetracycline-based inducer.	This study.
pPLtetO-FtsL	Derived from pJKR-L-tetO. Titrates synthesis of FtsL with tetracycline-based inducer.	This study.

Supplementary Table 6: System parameters

Parameter	Value	Units
K_M	600	cell^{-1}
$V_{\max}$	10	$\text{cell}^{-1}\text{min}^{-1}$
α_0	5.4	$\text{cell}^{-1}\text{min}^{-1}$
α_1	12.9	$\frac{\text{cell}^{-1}\text{min}^{-1}}{\text{mmol glc/g DCW/h}}$
F_I	1740	cell^{-1}
F_T	2000	cell^{-1}
f_{critical}	0.16	mmol glc/g DCW/h

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