# Towards a cell-level model to predict bacterial growth under antimicrobial perturbation

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## 1 Objective

**How can we include antimicrobial effects of drugs in bacterial growth models accounting for different mechanisms of action?**

Rather than including antibiotic action only empirically on population growth, we create a new layer of detail on the single cell level. This single cell level should simultaneously allow for the

a) mechanistic integration of antibiotic effects,

b) prediction of population growth.

Our approach is illustrated by simulating the evolution of population counts over time (time-kill curve data) for constant tetracycline exposure to *E. coli*.

## 2 Background

Bacterial cell cycle phases are defined by breakpoints at which either chromosome replication or cell septation starts or finishes (See figure 1).

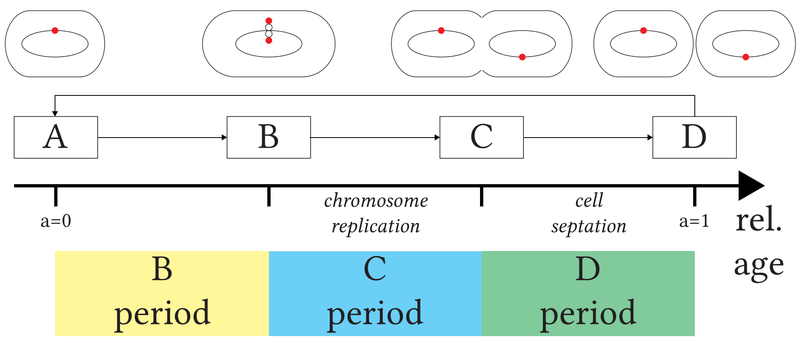



*Figure 1: Relative cell age $a\,[0-1]$ and cell cycle periods $B, C$ and $D$ during slow growth. Points of origin for chromosome replication as red dots.*

Problem: Multifork replication during fast growth (Mean replication time $\tau < C$ in min.)!
Solution: Cell state vector **Par** must explicitly include length of cell cycle periods: $B, C, D$

Additionally the biochemical composition and macroscopic parameters (e.g. cell size) of the reference cell change with varying growth rates [4].

**Fundamental observation [4, 7]:** Nutrient limitation leads to adaptation processes. The functional relationship between growth rate and cell state is invertible. However, many different nutrient media can lead to the same growth rate. This relation is not invertible.
We assumed, that bacteria adapt their cell state in the same way to certain antibiotics as they do to nutrient limitation.

A population of $N$ bacterial cells [CFU / mL], grows with rate constant $\mu$ in [1 / h], assuming no environmental limitations.

In transition between the typical phases of a bacterial population (lag, log, stationary and decay-phase) $\mu$ is not constant, but shifting:

$$\mu_{\text{lag}} \xrightarrow{\textbf{shift-up}} \mu_{\text{log}} \xrightarrow{\textbf{shift-down}} \mu_{\text{stationary}} \xrightarrow{\textbf{shift-down}} \mu_{\text{decay}}$$

If $d\mu/dt = 0$, the so called intensive properties of the reference cell (e.g. mass or ribosomes per cell) are constant, while the extensive ones (e.g. population mass or cell number) grow exponentially. We call this condition **balanced growth**.

## 3 Methodological approach

Antibiotic action modeled as a shift-down and determination of post-shift growth rate constant $\mu_2$.

$$\mu_1 \xrightarrow{\mathbf{Par}(\mu_1)} \mathbf{Par} \xrightarrow{E_{max}} \mathbf{Par} \xrightarrow{\mu_2(\mathbf{Par})} \mu_2$$

Starting with $\mu_1$ for $C_{drug} = 0$, $\mathbf{Par}(\mu_1)$ describes **Par** as a function of $\mu_1$, whereas $\mu_2(\mathbf{Par})$ returns the corresponding growth rate constant $\mu_2$. An $E_{max}$ model modifies **Par** according to $C_{drug}$. To describe the change of $\mu$ over time $t$ we introduced a transition rate constant $\alpha$ [1 / h].

$$d\mu/dt = \alpha(\mu_2 - \mu(t))$$

$$dN/dt = \mu(t)N(t)$$

### 3.1 Cell state vector

The values describing a reference cell with median age were summarized in the cell state vector **Par** (Figure 2).

$$\mathbf{Par} = \{nucl./prib.,\ aa/pol.,\ f_t,\ \beta_r,\ c_s,\ \Psi_s,\ \alpha_p,\ \beta_p,\ c_p,\ B,\ C,\ D\}$$

### 3.2 Cell state vector as a function of growth rate

The first four elements of **Par** were constant for a wide range of growth rates ($0.2 < \mu < 2.0$).

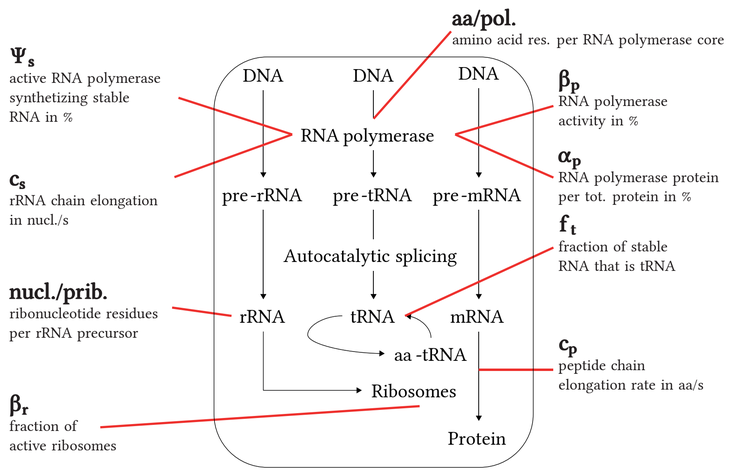



*Figure 2: Cell descriptors, their symbol and biological context.*

Adaptation processes were described by a continuous change of the last six elements of **Par** over $\tau = 60 \ln 2/\mu$ (Figure 3). Data refer to *E. coli* B/r [1] and were measured under balanced growth condition.

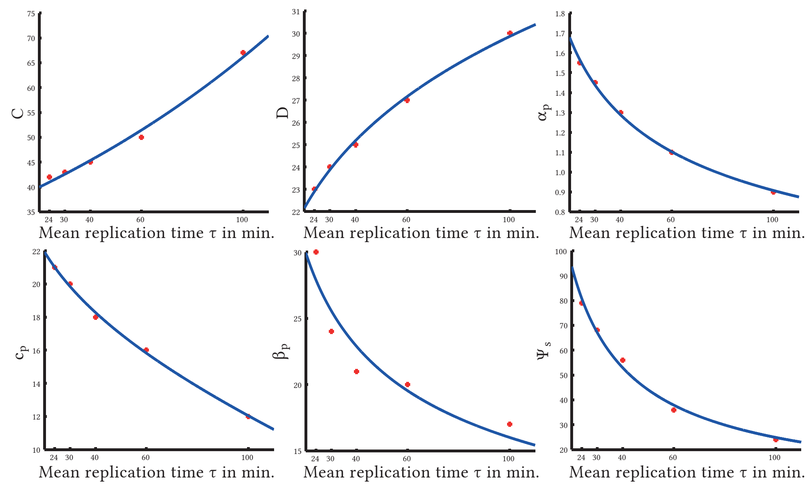



*Figure 3: Cell descriptors as functions of mean replication time $\tau$. Data as red crosses, fit in blue.*

### 3.3 Growth rate as a function of cell state vector

Empiric relation between growth-limiting parameters (Figure 3) and growth rate constant $\mu$ [8, 1].

$$\mu_2 = \frac{60}{[(nucl./prib.)(aa/pol.)/(1-f_t)]^{0.5}}(\Psi_s \alpha_p \beta_p \beta_r c_s c_p)^{0.5}$$

A consistency check ($\mu(\mathbf{Par}(\mu)) = \mu$) showed good correlation ($R^2 > 0.99$ for $0.2 \leq \mu \leq 2.0$).

### 3.4 Transition rate constant

A transit compartment model was used for cell cycle simulations. To determine the number of compartments $N_c$ and transit rate $\mu_{tran}$, we solved variance $\sigma^2 = N_c/\mu_{tran}^2$ and $\tau = N_c/\mu_{tran}$ by substitution - knowing the coefficient of variation $c_v = \sigma/\tau = 22\%$ [5], with standard deviation $\sigma$.

Knowing the age distribution during balanced growth [2], we initialized the pre-shift state (Figure 4, (1)). Each compartment had an index $i$, relative age $a_i$, frequency $F_i$, cell number $N_i$ and state $P_i \in \{B, C, D\}$. The post-shift initialization (Figure 4, (2)) was derived from this distribution (exact calculation not shown).

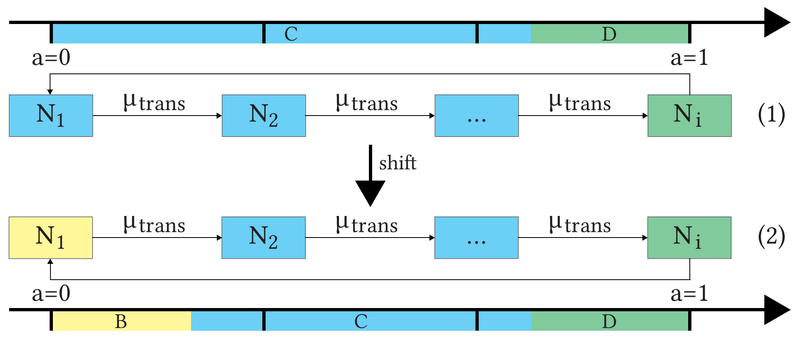



*Figure 4: Initial cell numbers in transit compartment model for pre- and post-shift condition. Here: Shift-down.*

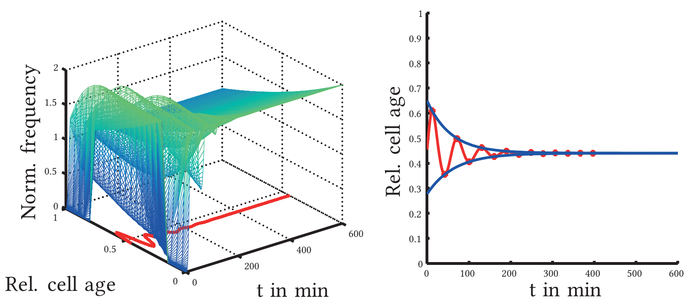



*Figure 5: Age distribution during shift-down ($\mu_1 = 2 \rightarrow \mu_2 = 1$). Left: Cell numbers for each compartment over time, in red mean age of the population. Right: In red mean age over time, in blue envelope fit.*

The mean age $\overline{a}$ of the population showed damped oscillation with two envelope functions $f(t) = a + b\exp(\alpha t)$ (See figure 5). Transition rate constant $\alpha$ was defined as the mean of both fits. Also $\alpha = \mu_2$ gave a good approximation [9].

## 4 Application

Tetracycline is a bacteriostatic antibiotic for most *E. coli* strains. It blocks the aa-tRNA binding site (A-site) at the 30S subunit of ribosomes, which leads to a halt during the elongation phase of translation. This results in a lower peptide chain elongation rate $c_{p_2}$, compared to pre-shift state $c_{p_1}$.

The extent of this inhibition was measured *in vitro* [6] and described by an inhibitory $E_{max}$ model. $C_{50}$ had to be corrected due to experimental differences between [6] and [3].

$$c_{p_2} = c_{p_1} - \frac{I_{max} C_{drug}^{\gamma}}{C_{50}^{\gamma} + C_{drug}^{\gamma}}$$

With $I_{max} = c_{p_1} \in \mathbf{Par}(\mu_1)$, $C_{50}$ = 0.0005 mg / mL, $\gamma$ = 1.42

Time-kill data were extracted from literature [3], $\mu_{max}(=\mu_1)$, $t_{lag}$ and $N_0$ were taken from control experiment.

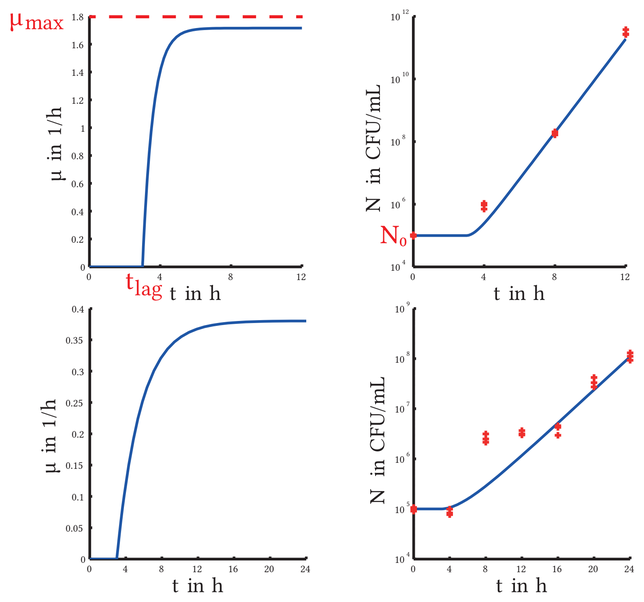
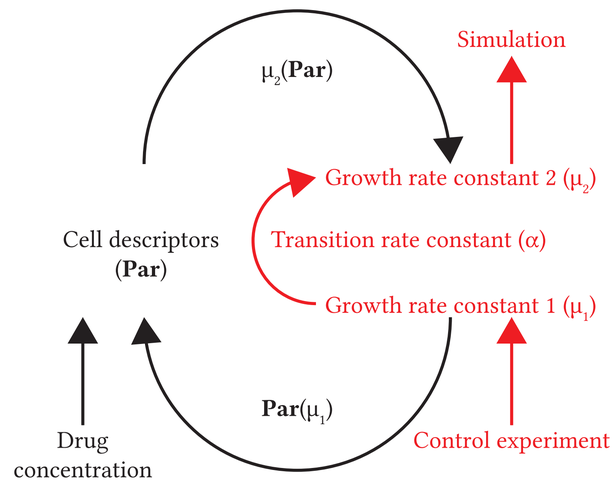



*Figure 6: **Upper panel:** Control experiment. Data in red, simulation in blue. Left: Input parameters $\mu_{max}$ and $t_{lag}$. Right: Time-kill curve. Note: Timespan 12 h.*
***Lower panel:** Constant exposure 0.004 mg / mL. Data in red, simulation in blue. Note: Timespan 24 h.*

Predictions were in agreement with experimental data.

## 5 Summary

Flow of information and algorithm to generate time-kill curve data.




1. Estimate $\mu_1$ from control experiment during balanced growth.
2. Compute corresponding cell state **Par** using $\mathbf{Par}(\mu_1)$.
3. Modify elements of **Par** according to $C_{drug}$.
4. Compute corresponding growth rate $\mu_2$ using $\mu_2(\mathbf{Par})$.
5. Compute transition rate constant $\alpha$.
6. Solve $d\mu/dt$ and $dN/dt$.

## References

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