Stochastic gene expression influences the selection of antibiotic resistance mutations

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Abstract

Bacteria can resist antibiotics by expressing enzymes that remove or deactivate drug molecules. Here, we study the effects of gene expression stochasticity on efflux and enzymatic resistance. We construct an agent-based model that stochastically simulates multiple biochemical processes in the cell and we observe the growth and survival dynamics of the cell population. Resistance-enhancing mutations are introduced by varying parameters that control the enzyme expression or efficacy. We find that stochastic gene expression can cause complex dynamics in terms of survival and extinction for these mutants. Regulatory mutations, which augment the frequency and duration of resistance gene transcription, can provide limited resistance by increasing mean expression. Structural mutations, which modify the enzyme or efflux efficacy, provide most resistance by improving the binding affinity of the resistance protein to the antibiotic; increasing the enzyme's catalytic rate alone does not contribute to resistance. Overall, we identify conditions where regulatory mutations are selected over structural mutations, and vice versa. Our findings show that stochastic gene expression is a key factor underlying efflux and enzymatic resistances and should be taken into consideration in future antibiotic research.

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1 Introduction

Efflux pumps and drug-inactivating enzymes can allow bacterial cells to evade the damage caused by antibiotic drugs. Bacterial pathogens with efflux and enzymatic resistances are ubiquitous and often are serious public health concerns (Li et al., 2015; World Health Organization, 2017). For instance, several gram-negative bacteria species with carbapenemase and ESBL-activities are listed as critical priority pathogens for research and development of new antibiotics by the WHO (World Health Organization, 2017).

Studies to quantify antibiotic resistance are routinely performed at the bacterial population level, i.e. through protocols such as minimum inhibitory concentration (MIC) or time-kill curves. However, the action of the drug occurs within the cell on the molecular level. Bridging these different scales remains a challenge.

The action of antibiotic drugs usually involves small numbers of molecules and binding sites, such that intrinsic stochasticity could have a significant effect on the dynamics. This noise impacts how we interpret bacterial assays that extract population-averaged behaviors, which normally ignore cell-to-cell heterogeneity. A recent study has suggested that many experimental phenomena, such as post-antibiotic effects, can be explained by these noisy within-cell dynamics (Abel Zur Wiesch et al., 2015).

Another source of randomness is in the mechanism of gene expression (Paulsson, 2005). Transcription typically occurs in bursts, a phenomenon often described by the so-called two-state or telegraph model of gene expression (Paulsson, 2005; Kumar et al., 2015; Lionnet and Singer, 2012). In this model, gene transcription switches stochastically between an active state with a constant rate of mRNA production, and an inactive state without transcription. Although certain genes may have complex transcription regulations, the two-state model is widely used when modelling both prokaryotes and eukaryotes due to its simplicity (Paulsson, 2005). The duration of active and inactive states can range from minutes to multiple cell generations (Lionnet and Singer, 2012; Hammar et al., 2014; So et al., 2011). This intrinsic stochasticity of gene expression is often the dominant source of randomness for gene-product numbers, and this separation of noise intensities has been used to perform mathematical analysis of gene-expression systems (Lin and Galla, 2016). While the expression level of resistance enzymes or efflux pumps generally correlates with the level of observed phenotypic resistance (Zwart et al., 2018), the effects of stochasticity on resistance remain unexplored.

Enzymatic resistances can be enhanced by regulatory or structural gain-of-function mutations, affecting the gene expression or the enzyme efficacy, respectively (Händel et al., 2014; Berrazeg et al., 2015; Yang et al., 2003; Blair et al., 2015). Both regulatory and structural mutations have been described for a number of antibiotic resistances (Händel et al., 2014; Toprak et al., 2012; Berrazeg et al., 2015). However, the selective conditions that favour one type of mutation over the other have not been fully explored.

Here, we provide a computational model that investigates the effects of stochastic gene expression on resistance and resistance evolution. Our model describes the dynamics of within-cell processes of the drug-target-efflux system and accounts for stochasticity in transcription and translation, as well as drug diffusion, binding, and removal. Using this model, we consider regulatory and structural resistance mutations to study their behaviour on the molecular, cellular, and population levels. Our goal is to explore how stochastic gene expression influences the survival and extinction of different types of resistance mutations under antibiotic treatments.

2 Model

2.1 Overview

We model a population of elongating and dividing cells in a boundless environment with an antimicrobial drug. The drug molecules can diffuse across cell membranes, and they influence the cell's death rate by binding to drug-specific targets. A drug efflux system, which is subject to stochastic gene expression, removes drug molecules from the cell. We focus on mutations that affect this system, either through regulatory effects or changes to the enzymatic efficacy.

Each cell is modelled as an independent agent, such that there are no between-cell interactions. To uphold independence, we assume a constant concentration of drug outside the cell. Spatial effects are ignored. The within-cell model takes the following processes into account: cell elongation and division; intracellular production of drug-target protein; entry of drug molecules into the cell; the interaction of target proteins with drugs and subsequent cell death due to antibiotics; and the transcription, translation and enzymatic activity of the resistance-mediating proteins. From here on we refer to the resistance-mediating protein as efflux protein, as it can remove drug molecules from the cell either as an efflux pump or drug-inactivation enzyme.

Each cell is described by seven discrete variables (shown in Table 1), which are updated stochastically using the adaptive tau-leaping Gillespie algorithm as implemented in the `adaptivetau` package in `R` (Cao et al., 2007). This is an approximation of the full stochastic simulation algorithm (SSA) (Gillespie, 1977). The interactions between the within-cell variables can be seen in the model schematic in Fig. 1 and are described in detail below. In addition, we model the elongation of the cell deterministically. All cell parameter values are reported in Supplemental Table S1.

Table 1: Within-cell variables

Variable	Description
ϕ	Free drug molecules in cell
P_{T}	Number of unbound targets
θ_{T}	Drug–target bound state
D_{E}	Expression activity of efflux gene (0 or 1)
M_{E}	mRNA transcribed by efflux gene
P_{E}	Efflux products translated from mRNA
θ_{E}	Drug–efflux bound state

2.2 Cell physiology and division

The cells we model here are *Escherichia coli*. We assume that the cells are cylindrical with cross-sectional diameter d. A newly-divided cell has length ℓ_0. The cell elongates exponentially until it reaches twice its length at birth (Campos et al., 2014), which is achieved in time interval t_G. At a time t after birth, the cell length (ℓ), surface area (A), and volume (V) satisfy

$$\begin{aligned} \ell(t) &= \ell_0 2^{t/t_G}, \\ A(t) &= \pi d \ell(t) + \frac{1}{2}\pi d^2, \\ V(t) &= \frac{1}{4}\pi d^2 \ell(t). \end{aligned} \tag{1}$$

For each cell, a small amount of zero-expectation Gaussian noise (standard deviation = 5%) is added to the mean generation time ($t_g \to \mathcal{N}[t_G, (0.05 t_G)^2]$) to desynchronise cell divisions and to increase cell-to-cell heterogeneity. Division happens instantaneously once the cell reaches twice its birth-length, and produces two equal-length daughter cells. All variables in Table 1 are

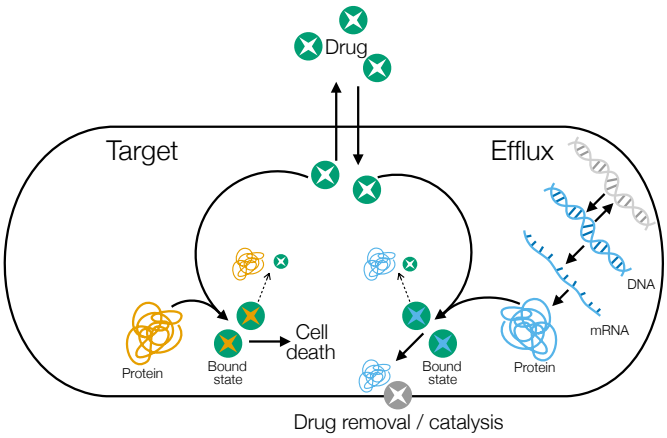

Figure 1: Model schematic. Each arrow corresponds to a discrete reaction, as described below. Dashed arrows indicate that drug–protein bound states may dissociate (depending on the drug used). Efflux proteins are translated from mRNA, which in turn is transcribed from DNA. The DNA can be active (blue) or inactive (gray), and can switch between these two states. Not shown is the constant production of target proteins, which we assume are constitutively expressed.

divided binomially between the two daughter cells upon cell division, with the exception of the DNA activity D_{E}, which is directly inherited by both daughters.

2.3 Target production

The target proteins usually fulfil essential functions in the cell, such as ribosomal subunits, RNA polymerase, DNA gyrase, and cell-wall components (Andersson and Hughes, 2010). The numbers per cell of several targets have been well-characterized and used for computational studies (Abel Zur Wiesch et al., 2015). The intracellular concentrations of these essential proteins may impact the cell's growth, for instance as in the case with ribosomes (Greulich et al., 2015). Therefore, we assume that these target proteins are subject to various intracellular regulations that maintain a stable and optimal concentration within the cell. This assumption is consistent with previous findings showing essential genes have low protein-expression noise (Silander et al., 2012). In the model this is achieved through constant production of targets over the duration of the cell's life. The production rate Γ is chosen such that, on average, the number of targets doubles in a generation. I.e., $P_{\mathrm{T}}(t_G) = P_{\mathrm{T}}(0) + \Gamma t_G = 2P_{\mathrm{T}}(0)$, such that

$$\Gamma = P_{\mathrm{T}}(0)/t_G. \tag{2}$$

As the half-life of bacterial proteins is usually on the scale of multiple hours (Larrabee et al., 1980), we neglect protein degradation relative to dilution-by-cell-division.

2.4 Efflux production

The transcription and translation of efflux proteins are explicitly modelled: we expect the expression of these non-essential genes to be more stochastic than that of the drug targets. The gene activity switches on(off) with a fixed rate $\Gamma_{\mathrm{On}}(\Gamma_{\mathrm{Off}})$, and therefore follows the telegraph process of gene expression (van Kampen, 2007). Once the gene is activated ($D_{\mathrm{E}} = 1$), mRNA is transcribed with a constant rate τ. The mRNA molecules degrade with rate γ. On average, b proteins are translated during the lifetime of each mRNA molecule. As above, we assume that these proteins do not decay but are diluted only by cell division. Given that these processes are intrinsically stochastic, a brief period of gene activation may not always produce efflux mRNA

and/or protein.

2.5 Drug diffusion, interaction, and efflux

The antibiotic drug outside of the cells is kept at a constant concentration c_{out}. The drug molecules diffuse through the cell envelope bi-directionally with a fixed diffusion rate σ. The overall diffusion rate is proportional to the surface area of the cell, $A(t)$. We use the stochastic version of Fick's law of diffusion for the bi-directional movement, such that transport is driven by a concentration gradient.

Once a drug molecule has entered the cell, it can either diffuse out, bind to its specific target, or be captured by an efflux protein. These binding events result in the formation of a drug–protein bound state. Binding occurs with the forward rate constant k_f, reflecting the binding affinity of target and efflux proteins. The actual binding rate also depends on the concentration of the drug and the proteins that interact inside the cell. Drug-binding to target and efflux are both considered reversible unless stated otherwise, and the bound states dissociate with backwards rate k_b. This event releases both the drug molecule and the protein into the cell. To compete efficiently with targets, efflux proteins should have at least the same or higher drug-binding rates than target proteins. Therefore we set the basal efflux binding rate to be the same as that of targets, making them indistinguishable for the drug.

The efflux protein has the capacity to remove the drug molecule once bound, through catalysis or transport, and this happens with rate k_{cat}. Alternatively the drug molecules can spontaneously deactivate when bound, releasing only the efflux or target protein to which it was bound, with rate k_d. For example, bound beta-lactam antibiotics can spontaneously deacylate and become inactive (Maveyraud et al., 1996).

All of the above reactions are summarised in Table 2.

Table 2: Within-cell reactions

Reaction	Rate	Description
$\emptyset \longrightarrow P_{\text{T}}$	Γ	Constant production of targets
$\emptyset \longrightarrow D_{\text{E}}$	$\Gamma_{\text{On}}(1 - D_{\text{E}})$	Switch on efflux gene
$D_{\text{E}} \longrightarrow \emptyset$	$\Gamma_{\text{Off}} D_{\text{E}}$	Switch off efflux gene
$D_{\text{E}} \longrightarrow D_{\text{E}} + M_{\text{E}}$	τD_{E}	Transcription of efflux mRNA
$M_{\text{E}} \longrightarrow \emptyset$	γM_{E}	Decay of efflux mRNA
$M_{\text{E}} \longrightarrow M_{\text{E}} + P_{\text{E}}$	$\gamma b M_{\text{E}}$	Translation of efflux product
$\emptyset \longrightarrow \phi$	$\sigma A(t) c_{\text{out}}$	Influx of free drug into cell
$\phi \longrightarrow \emptyset$	$\sigma A(t)\phi/V(t)$	Loss of free drug from cell
$\phi + P_{\text{T}} \longrightarrow \theta_{\text{T}}$	$k_f P_{\text{T}} \phi/V(t)$	Drug–target binding
$\theta_{\text{T}} \longrightarrow \phi + P_{\text{T}}$	$k_b \theta_{\text{T}}$	Dissociation of drug–target bound state
$\theta_{\text{T}} \longrightarrow P_{\text{T}}$	$k_d \theta_{\text{T}}$	Deacylation of drug in drug–target bound state
$\phi + P_{\text{E}} \longrightarrow \theta_{\text{E}}$	$k_f P_{\text{E}} \phi/V(t)$	Drug–efflux binding
$\theta_{\text{E}} \longrightarrow \phi + P_{\text{E}}$	$k_b \theta_{\text{E}}$	Dissociation of drug–efflux bound state
$\theta_{\text{E}} \longrightarrow P_{\text{E}}$	$k_d \theta_{\text{E}}$	Deacylation of drug in drug–efflux bound state
$\theta_{\text{E}} \longrightarrow P_{\text{E}}$	$k_{\text{cat}} \theta_{\text{E}}$	Catalysis/removal of drug

2.6 Cell death and MIC fraction

We combine two previously published models to implement a realistic death mechanism: the drug-induced death rate, $\delta(\rho)$, is an increasing function of the fraction of bound targets, ρ, but has a maximum value (Abel Zur Wiesch et al., 2015; Regoes et al., 2004). This fraction also correlates with the concentration of bound targets. The death rate should satisfy the following criteria:

1. $\delta(0) = 0$: In the absence of antibiotics the death rate is zero;

2. $\delta(\rho_{\mathrm{MIC}}) = \psi_{\max}$: When the external drug concentration is 1×MIC – which is the minimum concentration at which no bacterial growth is observed, the fraction of bound targets is given as ρ_{MIC}. The death rate at this value should equal the maximum growth rate in the absence of antibiotic ($\psi_{\max}$), such that the net growth rate is zero;

3. $\delta(1) = \psi_{\max} - \psi_{\min}$: When all targets are bound, the death rate is maximal. The net growth rate is then $\psi_{\min}$.

In our model, the death rate takes the sigmoidal form as in Regoes et al. (2004),

$$\delta(\rho) = \log(10)\frac{A\left(\frac{\rho}{\rho_{\mathrm{MIC}}}\right)^{\kappa}}{\left(\frac{\rho}{\rho_{\mathrm{MIC}}}\right)^{\kappa} - B}, \qquad \text{where } \rho = \frac{\theta_{\mathrm{T}}}{P_{\mathrm{T}} + \theta_{\mathrm{T}}}, \tag{3}$$

where the constants A and B are chosen such that the above criteria are satisfied. The values of $\psi_{\max}$ and $\psi_{\min}$ have been determined for multiple antibiotic compounds (Regoes et al., 2004). [Note that for Regoes et al. (2004) the number of cells, n, follows $n(t) = 10^{(\psi_{\max} - \delta(\rho))t} n(0)$, which accounts for the log(10) in Eq. (3).] At the cell level, the parameter ρ_{MIC} corresponds to the fraction of bound targets at which the cell has a 50% chance of surviving through its generation to replicate. Finally, the shape parameter κ in Eq. (3) determines the steepness of the sigmoidal response. For $\kappa \to \infty$, we recover the step function where death only occurs if the fraction of bound targets is greater than a threshold (in this case, the threshold would be ρ_{MIC}).

Through Eq. (3) we can relate the within-cell parameter ρ_{MIC} to the externally (population-level) measured MIC of the drug. Empirically, MIC is determined as the lowest drug concentration to prevent a small bacterial inoculum from proliferating overnight into visible density, sometimes quantified specifically as 80% or 90% growth reduction compared to a drug-free control (Arthington-Skaggs et al., 2002; Tunney et al., 2004). Here we define MIC more precisely as the drug concentration which results in a 50% survival probability of lineages founded by a single cell. Alternatively, one could assign the MIC as the concentration of drug at which the net growth rate is zero after a given time period; this is referred to as zMIC by Regoes et al. (2004). A further comparison could be made by computing the population size over an experimental duration, and assigning the MIC as the drug concentration which keeps the population size constant (no growth), or reduced to some small fraction of a drug-free control experiment. However, these simulations are extremely costly as many cells have to be tracked over a long period of time.

We determined values of ρ_{MIC} by simulating lineages emerging from individual wildtype cells that grow for 20 hours in a constant environment with $c_{\mathrm{out}} = 1\times\mathrm{MIC}$. This time interval is comparable to standard overnight MIC-experiments (Regoes et al., 2004). We then screened for ρ_{MIC}, defined as the highest value of bound target fraction where more than 50% of the progenitor cells and their lineages become extinct. This screening process is highlighted in Supplemental Fig. S1 for the drug ciprofloxacin, from which we find an MIC-fraction of 8.5%. Although this number may seem low, it could potentially be explained by the drug mechanism: ciprofloxacin binds to DNA-bound gyrases and fragments the bacterial chromosome via DNA doublestrand-breaks (DSBs) (Kampranis and Maxwell, 1998; Tamayo et al., 2009). Given that each *E. coli* cell has ~ 300 DNA-bound gyrases (Chong et al., 2014), this MIC-fraction suggests that a cell is likely to die when its chromosome is fragmented by over 20 simultaneous DSBs. The parameters are listed in Supplemental Table S2, and we repeat this screening procedure for rifampicin (Fig. S7) and cefotaxime (Fig. S8). Note that we only consider bactericidal drugs that introduce a drug-dependent death rate for each cell. I.e., the presence of drug has no impact on the cellular generation time.

2.7 Simulation algorithm

To simulate a population of cells over multiple generations, we use the following algorithm:

1. Assign each initial cell a unique set of parameter values;
2. Identify the cell with the earliest birth time;
3. Run the adaptive tau-leaping simulation algorithm for this cell until the cell dies, or it reaches its predefined generation time;
4. If the cell survived, perform cell division to create two new daughters;
5. Repeat from 2) until the experiment ends, no cells remain, or the number of cells is large enough that we can assume survival of the population for the duration of the experiment (100 unless otherwise stated).

We also describe the within-cell behaviour by a system of ODEs, as shown in the SM. We refer to these equations as the mean-field solution, as it does not take into account any stochasticity, and instead reflects the average dynamics of an infinite number of cells.

2.8 Model behavior

To test the validity of our model, we checked the distribution of target and efflux molecules across a large ensemble of wildtype cells growing in the absence of antibiotics (Supplemental Fig. S2). We found that the frequency of cells with active efflux DNA agrees with the prediction of the telegraph process ($\sim 2.5\%$), while the average number of efflux mRNA (0.04) and efflux proteins (4) per cell are within experimentally reported ranges (Moran et al., 2013; Cai et al., 2006; Taniguchi et al., 2010). The efflux protein copy number also follows a negative-binomial distribution, as predicted by the theory of these bursty dynamics (Paulsson and Ehrenberg, 2000). Finally, the number of target proteins per cell is normally distributed, which is expected given its constant production rate.

In Fig. 2 we show an example trajectory of the intracellular model variables in the absence and presence of an antibiotic (ciprofloxacin). Starting from a single wildtype cell, we track only one daughter cell after each division. After six generations we instate an external drug concentration, such that the drug can freely diffuse into the cell (Fig. 2A). The mean-field equations closely approximate the number of constantly-produced intracellular drug targets (Fig. 2B). The number and fraction of drug-bound targets is also well approximated in the mean-field limit (Fig. 2C,D). However, the bursty dynamics of the efflux system are not well captured by the deterministic approximation (Fig. 2E–H). Here a piecewise deterministic Markov process, which accounts for the gene-expression noise, would be a more appropriate approximation for this system (Lin and Galla, 2016).

Although cells could accumulate multiple efflux proteins during the period of gene activity (Fig. 2G), we did not observe two or more drug-bound efflux proteins at the same time (Fig. 2H). This results from two factors: the binding rate of drug is slow enough to disallow near-simultaneous formation of multiple drug–efflux bound states, while the catalysis rate is high enough that any efflux-bound drug molecule is quickly catalyzed. Put together, it shows that here the rate-limiting factor for removing drugs by efflux is not the catalysis step, but the drug-binding process (for this set of parameters).

2.9 Mutations

We consider seven classes of cells throughout this study: knockout (KO), wildtype (WT), three types of regulatory mutants and two types of structural mutants. Knockout (KO) cells lack efflux

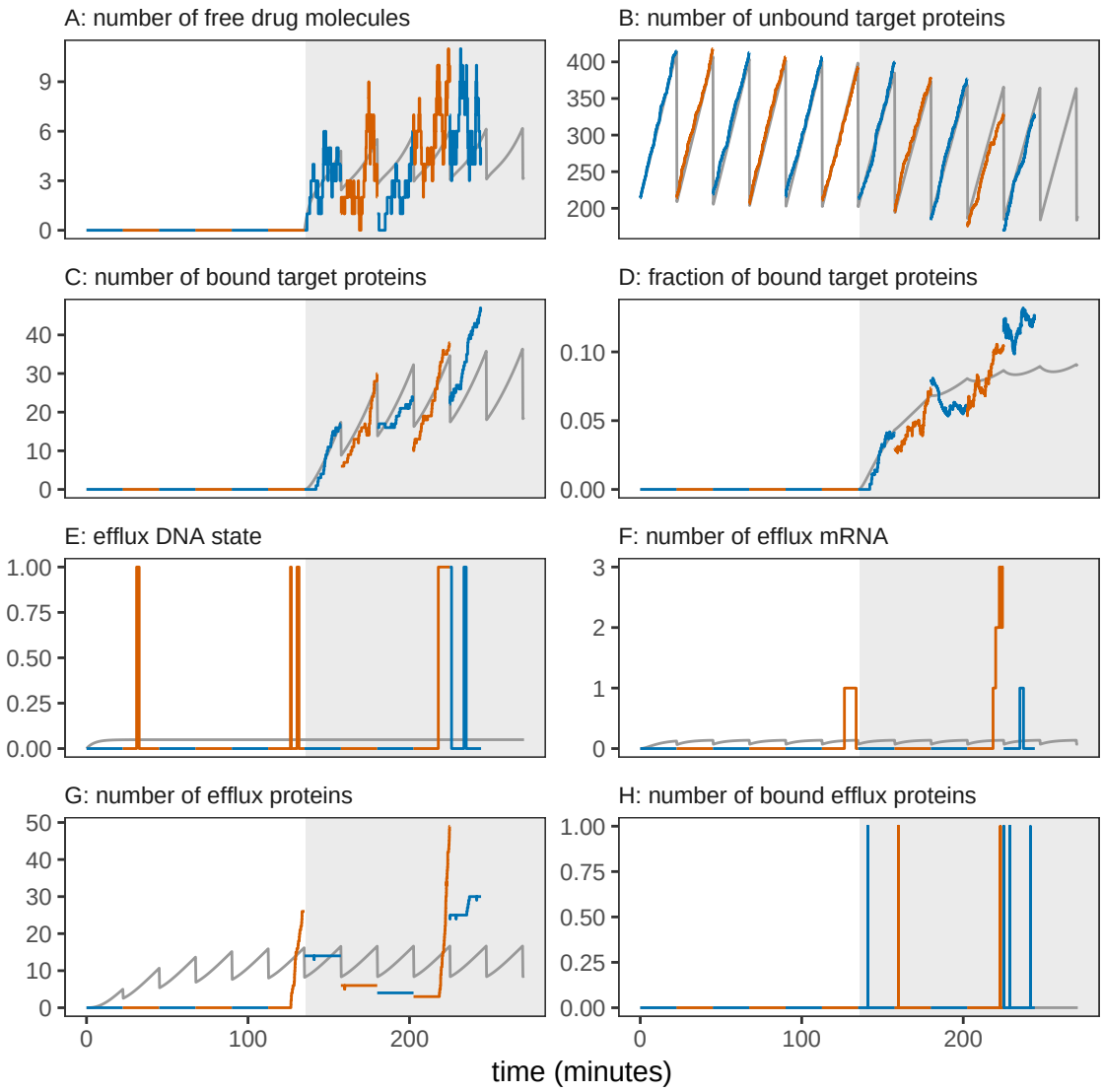

Figure 2: Intracellular variables in the absence (white background) and presence (grey background) of ciprofloxacin. Cell generations are distinguished by alternating line colors. Solid gray lines show the expected values based on mean-field solutions. The external drug concentration is set to zero for the first six generations, and then to $c_{\text{out}} = 1\times\text{MIC}$. The tracked cell lineage died after ~ 250 minutes. The panels show: A) number of free intracellular drug molecules; B,C) number of free and drug-bound target proteins, respectively; D) fraction of target proteins that are bound by the drug; E) stochastic expression of the efflux gene; F) number of efflux mRNA in the cell; G,H) number of free and drug-bound efflux proteins, respectively. Model parameters are listed in Supplemental Tables S1 and S2, but we double the DNA activation rate Γ_{On} to more clearly illustrate the stochastic gene expression dynamics in a shorter time window.

protein production. Wildtype (WT) cells have the basal level of efflux production. The effects of a mutation are characterized by a multiplicative parameter $\mu > 1$. The three types of regulatory mutants we consider are: REG-ON, which has an increased rate of gene activation ($\Gamma_{\text{On}} \to \mu\Gamma_{\text{On}}$); REG-OFF, which has longer periods of active transcription by reducing the inactivation rate ($\Gamma_{\text{Off}} \to \Gamma_{\text{Off}}/\mu$); and REG-BURST, which increases the translation rate or protein burst size from each translational event ($b \to \mu b$). The two types of structural mutants include STRUCT-BIND with improved binding affinity ($k_f \to \mu k_f$) to drug molecules and STRUCT-CAT with improved catalytic rates to break down drug molecules ($k_{\text{cat}} \to \mu k_{\text{cat}}$). The mutant cells also carry a cost, $0 < \nu \ll 1$, which is associated with their modified function. We assume this cost affects all metabolic processes in the cell, leading to a longer generation time, as well as slower

translation. We therefore implement $\Gamma \to (1-\nu)\Gamma$, $b \to (1-\nu)b$, and $\langle t_G \rangle \to \langle t_G \rangle / (1-\nu)$. The KO cell type carries a negative cost, as we assume it grows slightly faster for lacking efflux protein production completely.

3 Results

3.1 Lineage survival

We first simulate the survival probability of cell lineages when faced with a fixed antibiotic (ciprofloxacin) concentration. As well as considering different mutant classes, we also vary the mutant effect parameter μ. KO and WT classes are included as controls.

Four classes of mutant show distinct survival probability profiles (Fig. 3). REG-BURST mutants share an almost-identical profile to STRUCT-BIND, therefore the results for this class are relegated to the supplemental material (Supplemental Fig. S6). REG-ON mutants are more likely to survive at low drug concentrations (near the WT MIC) than any other mutant class, but their survival probabilities decline sharply with increasing drug concentrations (Fig. 3A). Death is more prevalent at low concentrations for REG-OFF mutants when compared to REG-ON, but the decrease in survival probability with concentration is slower (Fig. 3B). Ultimately, REG-ON and REG-OFF mutants require similar concentrations to eliminate the population. STRUCT-BIND mutants (and also REG-BURST) have much lower survival probabilities than regulatory mutants at low drug concentrations (Fig. 3C). However, a small fraction of STRUCT-BIND mutants can survive high drug concentrations beyond the simulated concentration range. STRUCT-CAT mutants, on the other hand, do not show any noticeable improvement in survival probability compared to WT or KO (Fig. 3D).

From Fig. 3 we interpolate IC50 and IC99.9 values, defined as the drug concentration for which the survival probability is 50% and 0.1%, respectively. These values represent the mutant prevention concentration (MPC) and minimum bactericidal concentration (MBC), respectively. Both regulatory mutations are characterized by successive increase in both IC50 and IC99.9 with increasing mutant effect. STRUCT-BIND shows a small increase in IC50, but significant increase in IC99.9 with increased binding rate. STRUCT-CAT has slightly lower IC50 and IC99.9 than WT, due to fitness cost of resistance and no obvious benefit of increasing the rate of catalysis. Conversely, KO has slightly higher IC50 and IC99.9 than WT due to its small fitness advantage. The values are reported in Supplemental Table S3.

For the cell lineages that became extinct in Fig. 3, we looked at their extinction times (Supplemental Fig. S3). Like the WT and KO cell types, the structural mutants and REG-BURST have their peak mean extinction time near the MIC. If extinction occurs at sub-MIC concentrations (which is a rare event, as shown in Fig. 3), it is most likely to happen at the beginning of the simulation when cell numbers are small. At $1\times$MIC, the extinction time diverges as each cell in the model has 50% chance of dying at this drug concentration (so that the expected lineage lifetime becomes infinite). As the drug concentration increases beyond the MIC, more drug diffuses into the cells and the death rate increases, leading to faster extinction of the cell lineages. Compared to WT and KO, both REG-ON and REG-OFF mutants show a shift of the peak extinction time towards higher concentrations with larger mutational effects. This corresponds to the shift in their respective MIC values, or IC50. Finally, we note that the STRUCT-BIND lineages of cells show a broad distribution (large variance) of extinction times at high drug doses.

Combining the data on survival probability and extinction times, our results suggest that REG-ON mutations provide intermediate but homogeneous resistance. This is characterized by the high survival probability at low drug concentrations, which declines quickly as the drug

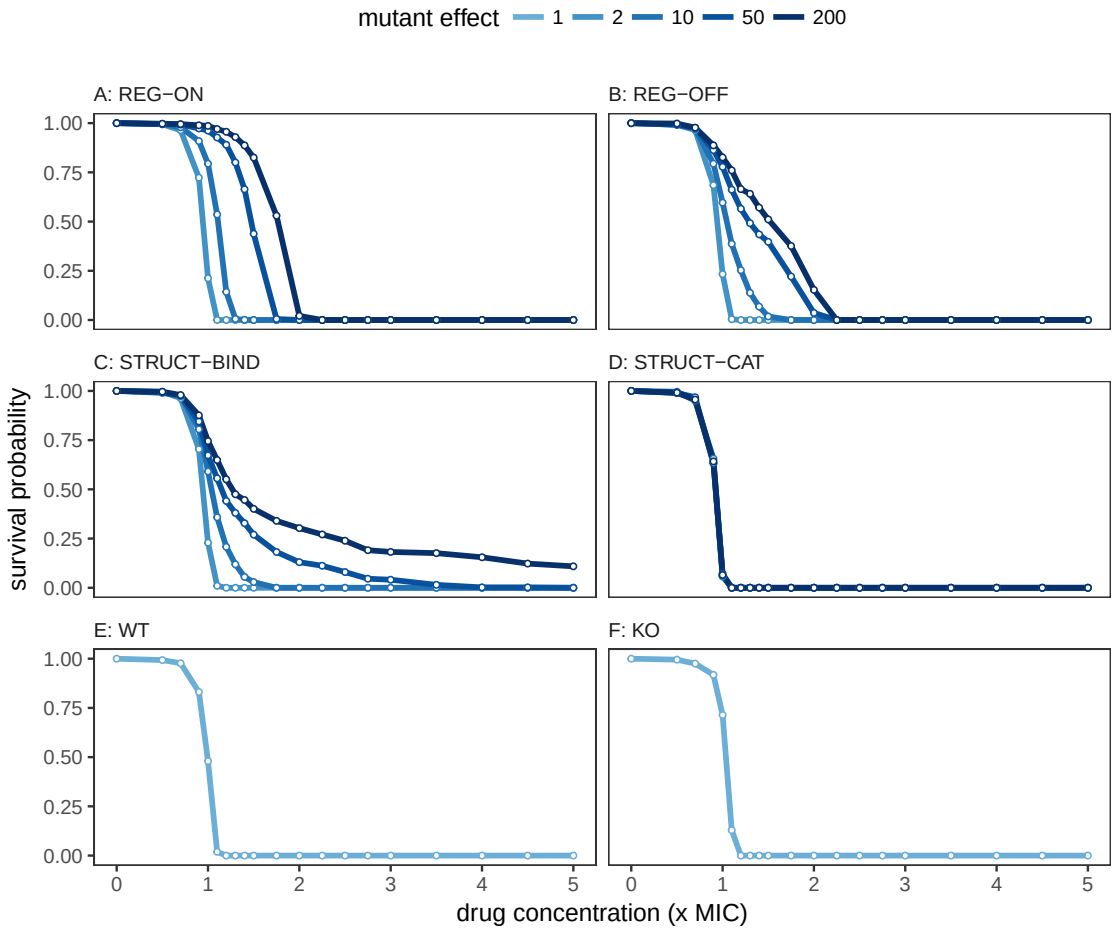

Figure 3: The survival probability of the different mutant classes across different concentrations of ciprofloxacin. Drug concentrations ranged from 0×MIC to 5×MIC. The mutant effects ranged from two- to 200-fold. Each data point is the mean of 1,000 individual simulations, with each simulation starting from one individual cell of the respective mutant class. Each simulation ends when either the population size reaches 100 cells (at which point extinction is extremely unlikely), all cells die, or we reach the end of the experiment at $t = 1,200$ minutes. Survival probability indicates the fraction of simulations with more than zero live cells.

concentrations increase until all cells eventually die. Resistance resulting from a REG-OFF mutation, however, seems heterogeneous. This is characterized by prominent deaths even at low concentrations but with some cell lines surviving through intermediate drug doses. The heterogeneity is even more pronounced in the STRUCT-BIND mutants: the low extinction times show that some cells are dying early, but the continued survival through intermediate and high doses suggest that a fraction of the cells is highly resistant. For STRUCT-BIND, the extreme resistance heterogeneity should be the combined result of low but highly stochastic gene expression and elevated binding rates of the resistance enzyme, such that the few cells with copies of the improved efflux protein are highly protected.

3.2 Growth rates

An alternative measure of mutant performance is through their population-level growth rates across different drug concentrations. As well as measuring the level of resistance conferred, these results can be directly compared with empirical observations. Following the protocol of Regoes et al. (2004), we simulated populations of cells for one hour and counted the number of cells at 10-minute intervals, before extracting the net population growth rate (Fig. 4).

Both regulatory mutants show similar dose-response profiles, which gives no indication of

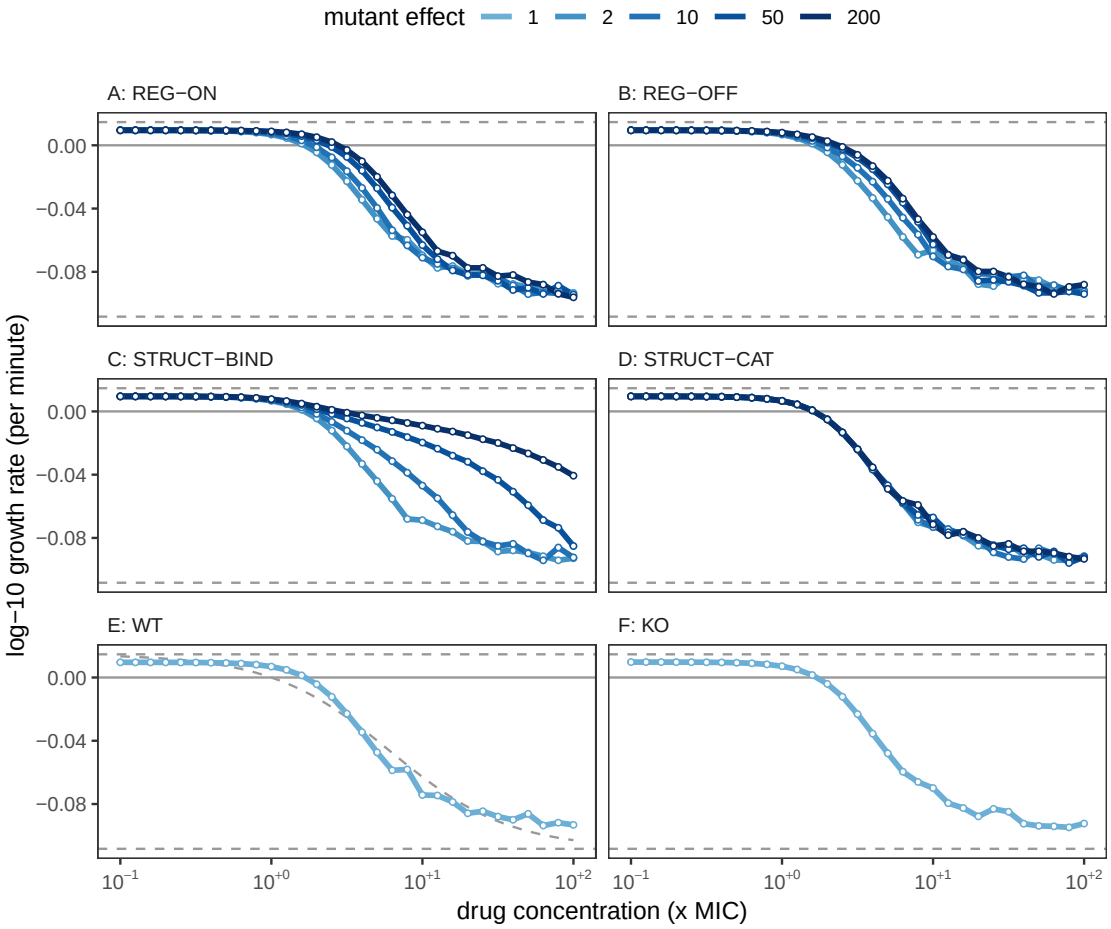

Figure 4: Population growth rates during the first hour of antibiotic exposure. For each mutant class and effect level, we simulated a population of 10,000 cells, and recorded the number of live cells at 10 minute intervals. From the corresponding time-kill curves (shown in Supplemental Fig. S4), we extracted the net growth rate as the linear regression coefficient between the base-10 logarithm of cell number and the sample time, as described by Regoes et al. (2004). Horizontal dashed lines are the maximum and minimum growth rates, $\psi_{\max}$ and $\psi_{\min}$. The dashed curve in panel E is the dose-response curve from Regoes et al. (2004).

the differences in their lineage survival probability. STRUCT-BIND mutants, however, generally maintain a higher net growth rate under high drug pressure when compared to the other cells types. This is in agreement with Fig. 3, where a few cell lineages are able to proliferate at these high treatment intensities. STRUCT-CAT, WT, and KO cells show the classical sigmoidal dose response, and the WT dose-response curve agrees well with the data reported by Regoes et al. (2004) (Fig. 4E). We note that the net growth rates at 1×MIC is higher than zero for all cell types. This is a result of initial population expansion during the first generations when the within-cell drug level is lower than the external drug concentration.

3.3 Heterogeneity and resistance

The number of efflux proteins per cell in REG-ON and REG-OFF mutants are nearly identical in mean for a given mutation effect μ (Fig. 5). However, these mutant classes have very different distributions of efflux protein number across the population. For REG-ON mutants the protein number distribution is unimodal such that cells are more homogeneous; with frequent transcription bursts (e.g. at $\mu = 50$ and 200) all cells produce some efflux proteins. REG-OFF mutants, in contrast, display a bimodal distribution of protein number: A large fraction of cells within the population do not produce any efflux protein, while some produce more than the corresponding

REG-ON mutant. This is the cause of REG-OFF cell death even at low drug concentrations, but with some survival at intermediate doses.

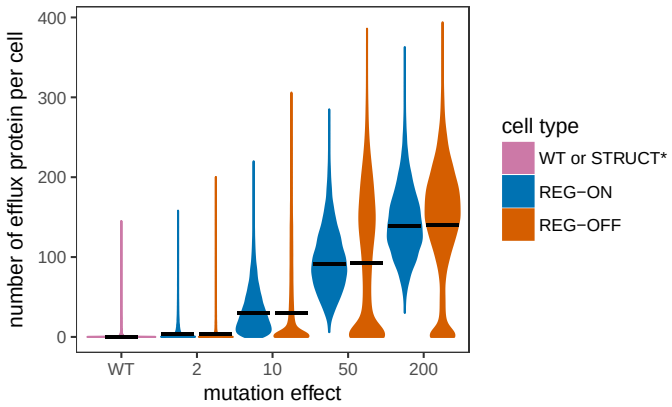

Figure 5: The distribution of efflux protein copy number per cell for regulatory mutants, across a range of mutant effects μ. Wildtype (WT) cells are included for comparison, and the expression of structural mutants is identical to WT. The mean protein copy numbers per cell are indicated by solid black lines. A population of 10,000 individual cells was simulated up to $t = 1,200$ minutes in the absence of drug, tracking only a single cell following each division event. Numbers are recorded immediately after cell division.

Although it is commonly accepted that increased mean expression of resistance enzymes can enhance resistance (Zwart et al., 2018), our results suggest that expression noise is also important for the population-level dynamics. Furthermore, regulatory mutations only provide limited resistance. For REG-ON and REG-OFF with 200× mutational effects, transcription is active for $\sim 80\%$ of the time. Therefore, resistance cannot be improved significantly by augmenting the transcription dynamics beyond this level. Further improvements in resistance then rely on increasing the translation rate (resulting in more proteins per mRNA) or by improving the efficacy of the efflux protein itself. This is consistent with previous findings showing that overexpressing the AcrAB efflux pumps can only increase ciprofloxacin-resistance by maximally six-fold (Yang et al., 2003).

For STRUCT-BIND mutants, the efflux protein number distribution is very similar to that of the wild type as there are no changes in expression (apart from the small cost of carrying the mutation). Therefore, the majority of STRUCT-BIND cells in a population contain no efflux protein. However, those cells that do contain efflux proteins have a high level of resistance, due to the high efficacy of these efflux pumps. This is the source of heterogeneity in the STRUCT-BIND mutant population.

To investigate this further, we consider the survival for a STRUCT-BIND mutant, but with different levels of gene expression stochasticity (Fig. 6). Concretely, we increase the frequency of gene activation ($\Gamma_{\mathrm{On}} \to \alpha\Gamma_{\mathrm{On}}$ for $\alpha > 1$), and correspondingly decrease the efflux protein translation rate $\gamma b \to \gamma b/\alpha$. In this way, we maintain the mean number of efflux proteins per cell (Fig. 6A) but alter the expression noise.

By reducing the gene expression noise, we note an increased IC50, but decreased IC99.9 (Fig. 6B). For low expression noise (large α), the survival profile now resembles that of REG-ON mutants (see Fig. 3A). Although a higher drug concentration is required to inhibit mutant proliferation, mutants can be eliminated at lower bactericidal concentrations. With reduced expression noise the efflux protein number in cells becomes more uniform and cells with extreme protein abundances disappear, making resistance more uniform across the cell populations.

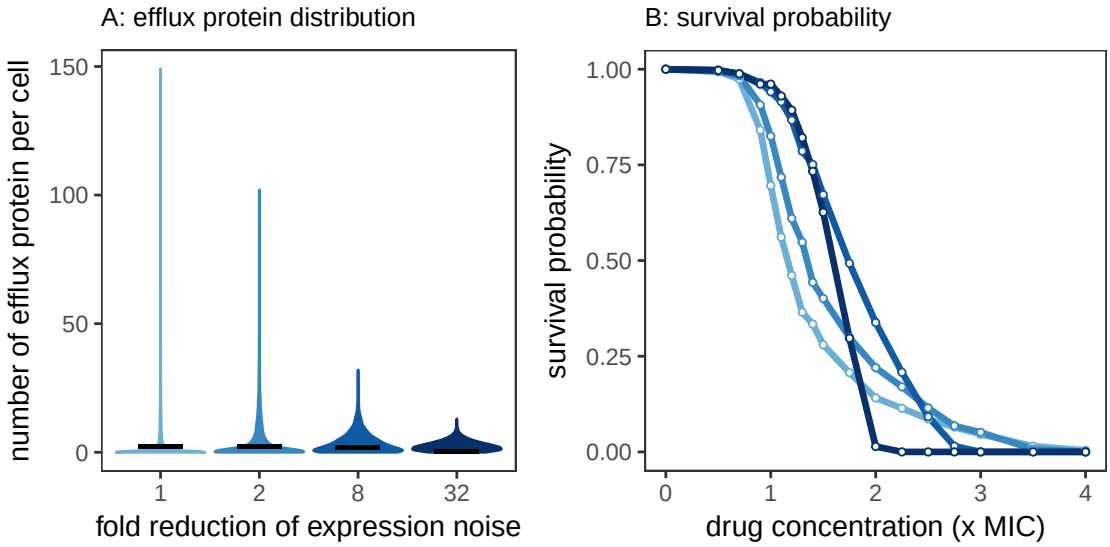

Figure 6: Controlling gene expression stochasticity in STRUCT-BIND mutants. A) Efflux protein number distributions across 10,000 cells with increased gene activation rate Γ_{On} and decreased translation rate γb. Solid black bars are the mean number of efflux protein per cell. B) Survival probability of STRUCT-BIND mutants with effect $\mu = 50$, with varying expression noise ($\alpha \in \{1, 2, 8, 32\}$). Colours correspond to the expression noise in panel A.

3.4 Survival after antibiotic pulse

The survival and extinction time properties of the different mutants are expected to affect the outcome of pulsed antibiotic treatment, which reflects the situation in a patient where drugs are not administered in a manner that maintains a constant concentration. Therefore we simulated pulsed treatments where cells are exposed to a one-time dose of antibiotic for a limited time period. For simplicity, the drug dose is expressed as a step-function without more-detailed pharmacokinetics. We then measured the survival probability for each mutant class across different treatment durations and concentrations (Fig. 7). This survival probability can also be interpreted as the genetic diversity, as it measures the fraction of unique lineages which survive the treatment.

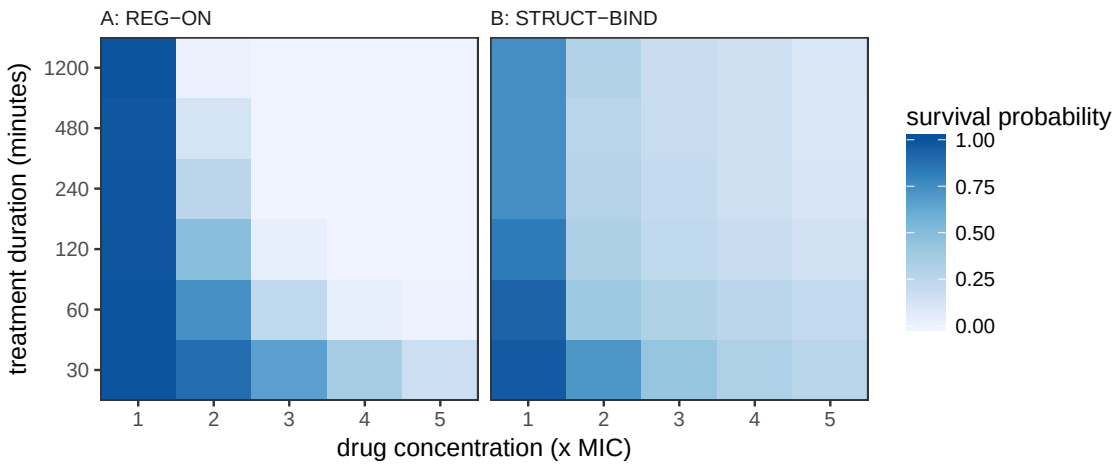

Figure 7: Survival probability after exposure to a pulse of antibiotics. The survival probability is indicated by color scale. The drug dose is applied as a step-function with constant external drug concentration during the pulse, and $c_{\mathrm{out}} = 0$ for the remainder of the experiment up to $t = 1,200$ minutes. Simulations are performed as in Fig. 3. The mutant effect is $\mu = 200$.

REG-ON mutants have the highest survival probability at low drug concentrations ($c_{\text{out}} \leq 2\times$MIC) or with brief drug pulses (e.g. $t = 30$ minutes), as shown in Fig. 7A. This is due to their long extinction times that allow regulatory mutants to outlast drug concentrations beyond their IC99.9. STRUCT-BIND cells survive at higher concentrations with long drug pulses (Fig. 7B), consistent with their high IC99.9 values. REG-OFF mutants perform similarly to REG-ON mutants, while STRUCT-CAT, KO, and WT cells show low levels of survival (Supplemental Fig. S5).

4 Discussion

Efflux pumps and drug-inactivating enzymes cause some of the most important clinical resistances (Li et al., 2015; World Health Organization, 2017). Overexpression of efflux pumps is also associated with virulence and enhanced mutation rates (Wang-Kan et al., 2017; El Meouche and Dunlop, 2018). Although stochasticity is inherent to gene expression and underlies vital cell functions, its effects on antibiotic resistance have not been systematically addressed.

To study this, we constructed an efficient agent-based model that bridges the scales between within-cell molecular processes and population-level dynamics. We considered different classes of regulatory and structural mutations that enhance resistance, and evaluate their performances under different drug pressures. Previous models have utilized drug–target binding processes to help explain population-level phenomena such as persistence (Abel Zur Wiesch et al., 2015). Our model extends this approach by adding enzymatic reactions. Furthermore, unlike previous deterministic models, our stochastic approach explicitly captures the noise at each step of the relevant biological processes, allowing us to dissect the effects of each source of noise on bacterial behaviour.

Regulatory mutations that increase either transcription frequency (REG-ON) or duration (REG-OFF) by the same factor have the same mean expression. Thus, a mean-field deterministic model would predict equal levels of resistance. However, increasing the duration of transcription does not reduce the noise of gene activation, leading to a subpopulation of cells with little or no resistance enzymes which remain drug-susceptible. This is not observed in the mutants with frequent gene activation, which instead show a homogeneous population of cells with equal resistance.

Structural mutations improve the biochemical properties of the resistance enzyme, either by increasing the binding rate to the drug (STRUCT-BIND) or the catalysis rate (STRUCT-CAT). While many structural mutations may influence both binding and catalysis simultaneously, here we disentangle them to elucidate their relative importance in resistance. We find that improving the binding rate has a significant effect on resistance, while improving the catalysis rate has no effect. Catalysis may play a role if drug diffusion or binding occur extremely fast, at which point the efflux proteins are essentially all drug-bound and catalysis becomes rate-limiting. Our findings, however, suggest that drug binding is the rate-limiting step.

In general, high regulatory noise results in heterogeneity in phenotypic resistance whereas low noise results in homogeneous resistance. By controlling the gene-expression noise, we show that while noise reduction may facilitate bacterial elimination of mutants by reducing their IC99.9, it may also exacerbate mutant inhibition by increasing their IC50. This is relevant for exploring the clinical potential of treatments which modulate gene-expression noise. Such noise-modulating chemicals have, for example, recently shown promising effects on reactivating HIV from latency, a process that relies on high gene expression noise (Dar et al., 2014). The success of HIV-latency modulators has provided a new concept in drug discovery and we envision that a similar approach may be tested to regulate resistance in bacteria.

In clinical antibiotic treatments, the drug concentration is not maintained at a consistently high level, as opposed to laboratory experiments. Therefore, the survival time until pathogens go extinct under drugs becomes relevant. Regulatory mutants have long survival times even at high concentrations, which leads to high survival probabilities as long as the drug is applied in a brief pulse. Thus, regulatory mutants could be more clinically problematic than structural mutants despite having lower IC99.9. This outcome will not be reflected in standard experimental protocols based on constant concentrations such as standard MIC-measurements. Therefore, a comprehensive understanding of mutant dynamics requires more detailed assessment methods for cell death and growth across different antibiotic concentrations.

Our drug–target model is based on ciprofloxacin, gyrase, and the AcrAB efflux pump. This system has the advantage of relatively few drug targets per cell and drug that acts at low concentrations, which reduces computation time. We parametrized our model based on previous experimental findings. It can accurately reproduce cell population dynamics under various drug concentrations as well as within-cell protein distributions under stochastic expression (Fig. 4E, Supplemental Fig. S2B). We therefore expect our model and its findings to be qualitatively robust.

Given that stochastic gene expression is a general mechanism in cell biology, our findings may also offer insights into general bacterial adaptation to changing environments. One example is the evolutionary reproducibility of adaptation via gene overexpression: in certain biological systems, regulatory mutations seemingly take precedence over structural mutations and vice versa (Toprak et al., 2012; Blank et al., 2014; Lind et al., 2015). Here we show that stochastic gene expression has temporal and heterogeneous effects on regulatory versus structural mutants, which helps explain why certain mutations could be advantageous.

In conclusion, the dynamics of gene expression could have a strong impact on the efficacy, and therefore the evolution, of resistance mutations. In particular, the intrinsic stochasticity of gene expression is a crucial determinant of evolutionary success. The framework we developed in this study now opens further opportunities to assess the impact of stochastic gene expression on bacterial adaptation, and the impact of molecular biology on evolution by extension.

5 Supplementary material

Tables S1-S3, Figs. S1-S8, and the description of the mean-field model can be found in the Supplementary Material. All code and generated data can be found at the github repository `https://github.com/ashcroftp/StochasticGeneExpression2019`. This will be archived on Zenodo and made publicly available prior to publication.

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Supplementary Material

Stochastic gene expression influences the selection of antibiotic resistance mutations

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S.I Mean field model

From the reactions in Table 2 of the manuscript, we can construct a mean-field ordinary differential equation (ODE) model:

$$\dot{P}_{\mathrm{T}} = \Gamma - k_f \frac{P_{\mathrm{T}}\,\phi}{V(t)} + k_b \theta_{\mathrm{T}}, \tag{S1a}$$

$$\dot{D}_{\mathrm{E}} = \Gamma_{\mathrm{On}}(1 - D_{\mathrm{E}}) - \Gamma_{\mathrm{Off}} D_{\mathrm{E}}, \tag{S1b}$$

$$\dot{M}_{\mathrm{E}} = \tau D_{\mathrm{E}} - \gamma M_{\mathrm{E}}, \tag{S1c}$$

$$\dot{P}_{\mathrm{E}} = \gamma b M_{\mathrm{E}} - k_f \frac{P_{\mathrm{E}}\,\phi}{V(t)} + (k_b + k_d + k_{\mathrm{cat}})\theta_{\mathrm{E}}, \tag{S1d}$$

$$\begin{aligned}\dot{\phi} &= \sigma A(t)\left[c_{\mathrm{out}} - \frac{\phi}{V(t)}\right] - k_f \frac{(P_{\mathrm{T}} + P_{\mathrm{E}})\phi}{V(t)} \\ &\quad + k_b(\theta_{\mathrm{T}} + \theta_{\mathrm{E}}),\end{aligned} \tag{S1e}$$

$$\dot{\theta}_{\mathrm{T}} = k_f \frac{P_{\mathrm{T}}\,\phi}{V(t)} - (k_b + k_d)\theta_{\mathrm{T}}, \tag{S1f}$$

$$\dot{\theta}_{\mathrm{E}} = k_f \frac{P_{\mathrm{E}}\,\phi}{V(t)} - (k_b + k_d + k_{\mathrm{cat}})\theta_{\mathrm{E}}. \tag{S1g}$$

For the behaviour shown in Fig. 2 of the manuscript, these equations are integrated until time t_G, after which the molecule numbers are halved to represent dilution due to cell division.

S.II Supplemental tables

Model parameters are given in Table S1, and drug specific parameters are given in Table S2. IC50/IC99.9 values for ciprofloxacin treatments can be found in Table S3.

S.III Supplemental figures

Parameter screening for ciprofloxacin is shown in Fig. S1. Molecule distributions are shown in Fig. S2. Extinction times are shown in Fig. S3. Time-kill curves are shown in Fig. S4. Survival probabilities following pulsed drug treatment are shown in Fig. S5. Results for REG-BURST mutants are shown in Fig. S6. Parameter screening for rifampicin is shown in Fig. S7. Parameter screening for cefotaxime is shown in Fig. S8.

Table S1: Summary of parameter values. [a]Weighted average of reported values. [b]$t_G = \log(2)/(\log(10)\psi_{\max})$. [c]Inactive time reported as 0.5–3,000 min. [d]Active time reported as 5–60 min. [e]Examples range from 5–40.

Parameter	Value	Description
d	934 nm [1]	Diameter of cell (constant)
ℓ_0	3.4 μm [2]	Length of newborn cell
$\psi_{\max}$	0.80 hour^{-1} [3][a]	Maximum population growth rate
$\psi_{\min}$	See Table S2	Minimum population growth rate
t_G	22.5 min [3][b]	Generation time
Γ	See Eq. (2) of the manuscript	Rate of target production
Γ_{On}	0.2 min^{-1} [4,5][c]	Rate of efflux gene activation
Γ_{Off}	0.005 min^{-1} [4,5][d]	Rate of efflux gene inactivation
τ	0.42 min^{-1} [6]	Transcription rate
γ	0.14 min^{-1} [7]	mRNA decay rate
b	22.5 [8][e]	Burst size (products per mRNA)
c_{out}	See Table S2	Outside drug concentration
σ	See Table S2	Drug diffusion rate
k_f	See Table S2	Binding rate of drug to target/efflux
k_b	See Table S2	Dissociation rate of drug–protein bound state
k_d	See Table S2	Deacylation rate of protein-bound drug
k_{cat}	See Table S2	Catalysis/removal rate of drug
MIC	See Table S2	Minimum inhibitory concentration
ρ_{MIC}	See Table S2	MIC fraction of bound targets
κ	See Table S2	Shape parameter
μ	> 1	Mutant effect
ν	5%	Cost of mutation

[1] Ouzounov et al. (2016); [2] Campos et al. (2014); [3] Regoes et al. (2004); [4] Lionnet and Singer (2012); [5] Hammar et al. (2014); [6] Golding et al. (2005); [7] Bernstein et al. (2002); [8] Thattai and Van Oudenaarden (2001).

Table S2: Drug parameters extracted from the literature. [a]Value is based on AcrB efflux pump. [b]Other drugs which target penicillin-binding proteins bind irreversibly, so we assume the same is true for cefotaxime. [c]Values chosen according to Fig. S1. [d]Values chosen according to Fig. S7. [e]Values chosen according to Fig. S8.

Drug	Ciprofloxacin	Rifampicin	Cefotaxime
Target	DNA Gyrase	RNA polymerase	Penicillin-binding protein 3
Number of targets	300 [1,2]	11,400 [3]	200 [4]
MIC (μg ml^{-1})	0.03 [5]	8.00 [5]	0.06 [6]
Molar mass (g mol^{-1})	331.347	822.953	455.46
Diffusion rate (m s^{-1})	2.0×10^{-11} [3]	2.0×10^{-11}	6.0×10^{-8} [7]
Binding rate (M^{-1} s^{-1})	3.6×10^{4} [8]	1.2×10^{6} [3]	4.6×10^{4} [9]
Dissociation rate (s^{-1})	3.0×10^{-4} [8]	1.2×10^{-3} [3]	0.0 [3][b]
Catalysis rate (s^{-1})	10.0 [7][a]	10.0 [7][a]	2.5 [10]
Deacylation rate (s^{-1})	–	–	2.5×10^{-4} [11]
Min. pop. growth rate (h^{-1})	-6.5 [5]	-4.3 [5]	-4.0 [12]
Shape (κ)	3.0[c]	20 [d]	0.0[e]
ρ_{MIC}	0.085[c]	0.28[d]	0.93[e]

[1] Chong et al. (2014); [2] Stracy et al. (2019); [3] Abel Zur Wiesch et al. (2015); [4] Weiss et al. (1997); [5] Regoes et al. (2004); [6] Nath et al. (1995); [7] Nagano and Nikaido (2009); [8] Kampranis and Maxwell (1998); [9] Adam et al. (1997); [10] Saves et al. (1995); [11] Terrak et al. (1999); [12] Xuan et al. (1997).

Table S3: Profiles of resistance to ciprofloxacin. The IC50 and IC99.9 values are expressed as multiples of the MIC for the WT strain. These values are extracted from Fig. 3 of the manuscript using linear interpolation, and are defined as the drug concentrations at which 50% and 99.9% of the lineages from the initial bacterial inoculum are killed.

Mutant class	Mutant effect (μ)	IC50	IC99.9
WT	1	1.0	1.2
KO	1	1.0	1.2
REG-ON	2	0.9	1.1
	10	1.1	1.4
	50	1.5	2.0
	200	1.8	2.2
REG-OFF	2	0.9	1.2
	10	1.0	1.7
	50	1.3	2.2
	200	1.5	2.2
REG-BURST	2	0.9	1.1
	10	1.0	1.7
	50	1.2	4.3
	200	1.3	> 5
STRUCT-BIND	2	0.9	1.2
	10	1.0	1.7
	50	1.1	4.5
	200	1.3	> 5
STRUCT-CAT	2–200	0.9	1.1

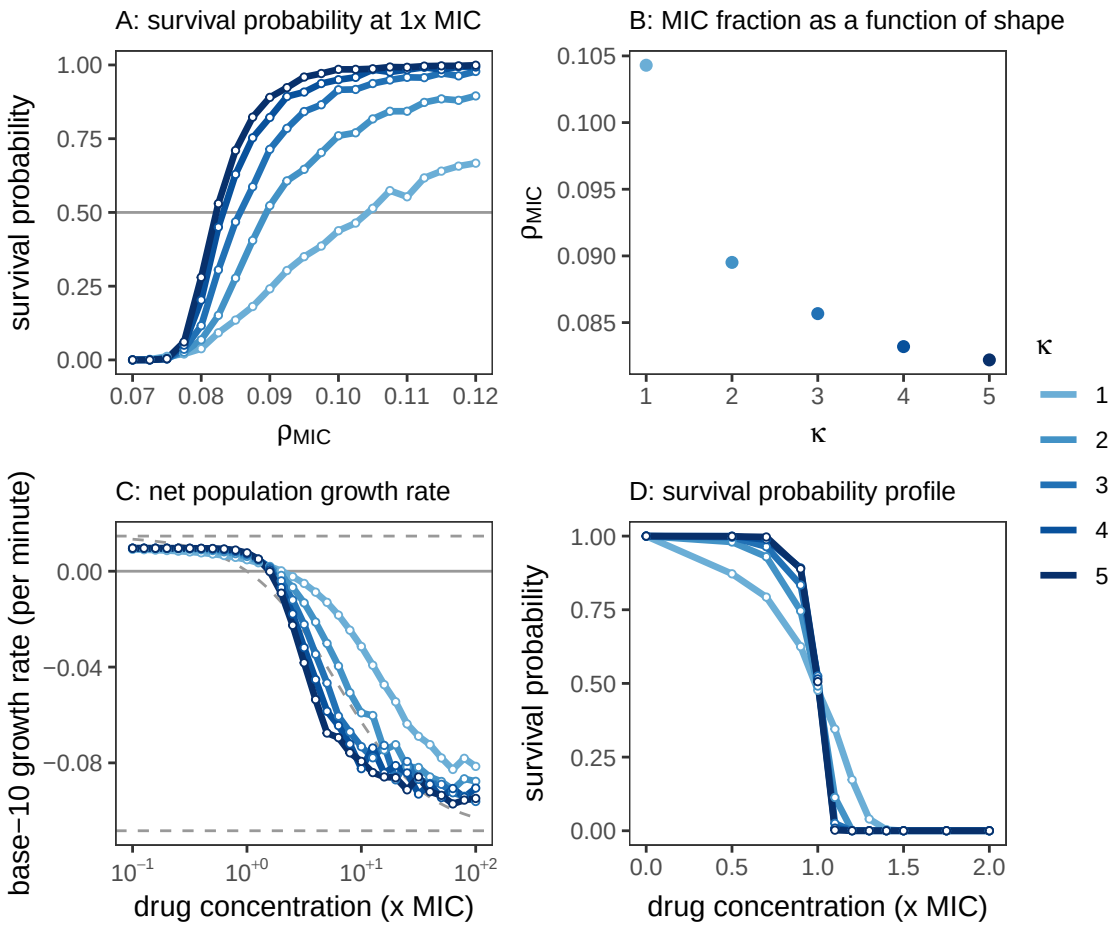

Figure S1: Parameter screening for ciprofloxacin. A) We calculate the survival probability for 1,000 lineages initiated from a single cell at 1× MIC while varying the MIC fraction of bound targets (ρ_{MIC}) and the shape parameter κ. B) From panel A we interpolate the value of ρ_{MIC} which gives a survival probability of 50%. C) For each pair of κ and ρ_{MIC} in panel B, we simulate a population of 10,000 cells under different drug pressure, and extract the net population growth rate. This can be compared with the maximum and minimum reported rates (dashed horizontal lines), and the growth curve reported by Regoes et al. (2004) (dashed curve). D) For each pair of κ and ρ_{MIC} in panel B, we calculate the survival probability of 1,000 lineages initiated from a single cell under different drug pressure.

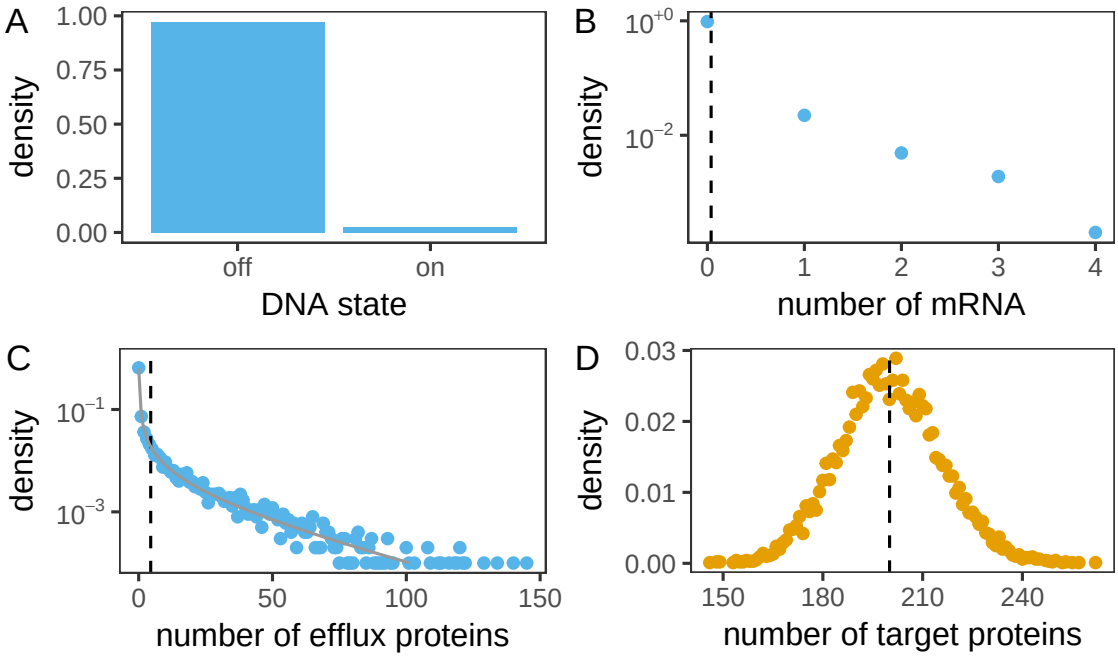

Figure S2: The distribution of efflux and target states across 10,000 cells immediately after cell division. These are sampled after $t = 1,200$ minutes in the absence of drugs. The panels show: A) expression state of the efflux gene; B) distribution of efflux mRNA across the population of cells; C) number of efflux proteins per cell, which fits to a negative binomial distribution (grey line); D) number of target proteins (gyrase) per cell. Parameters are based on the targets of ciprofloxacin, and can be found in Tables S1 and S2.

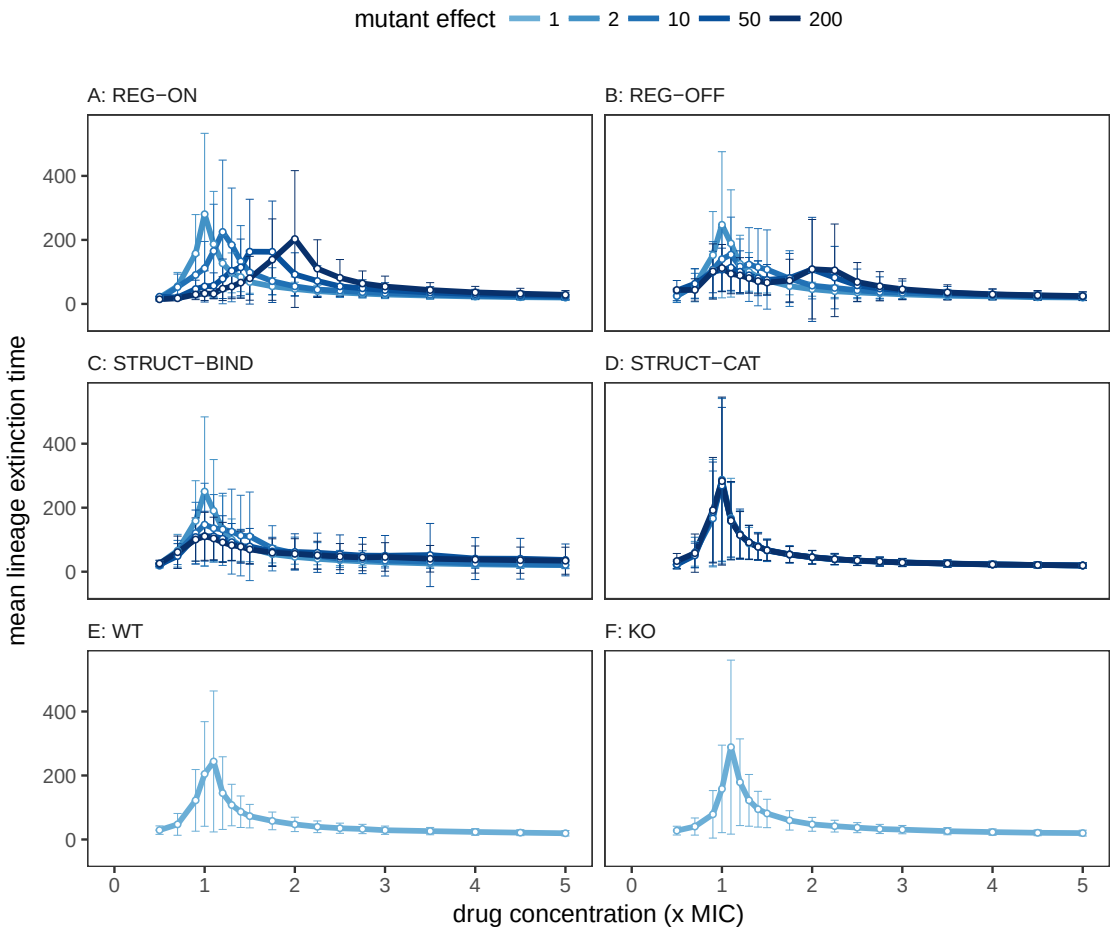

Figure S3: Mean extinction times of lineages of the different mutant classes. These are extracted from the same data as Fig. 3 of the manuscript. Error bars indicate the standard deviation of the extinction times.

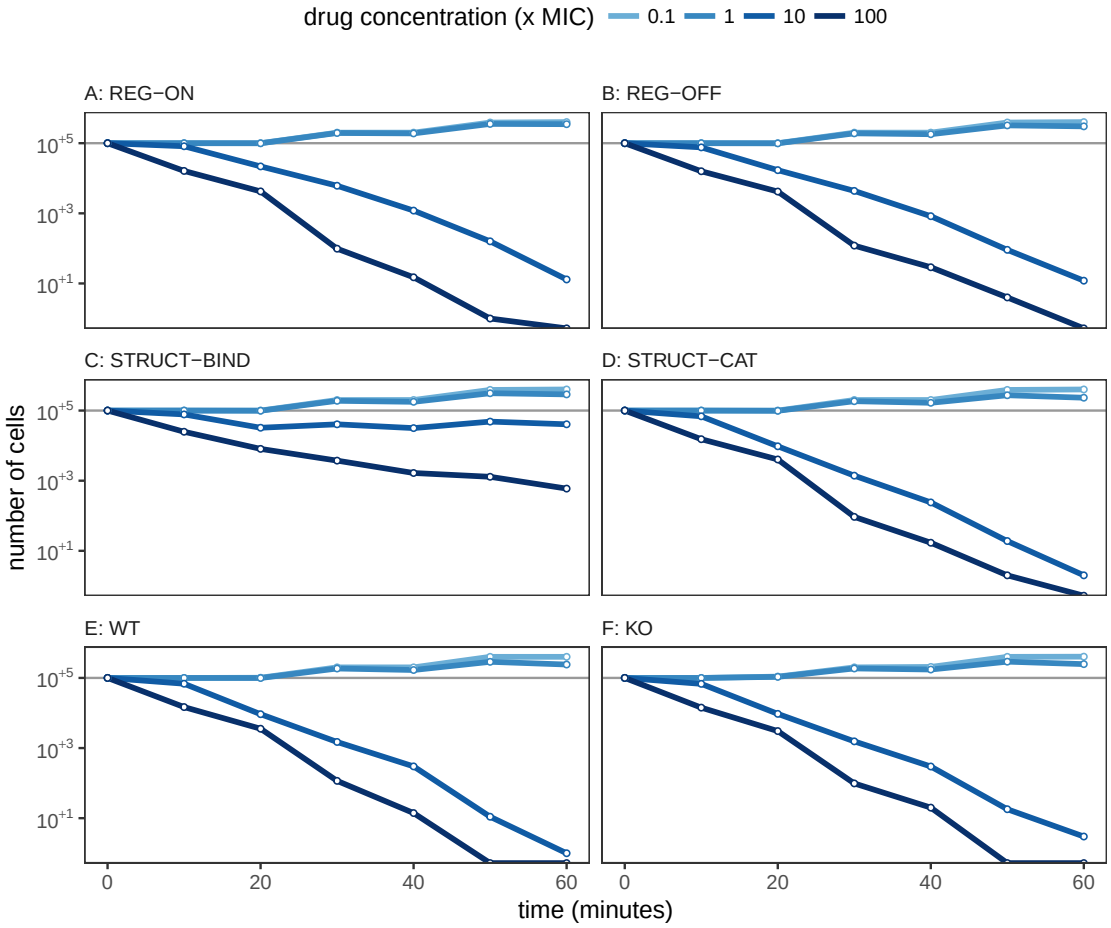

Figure S4: Time-kill curves, from which the growth rates in Fig. 4 of the manuscript are extracted. Here we only show mutant effect parameters $\mu = 200$ (for REG-ON, REG-OFF, STRUCT-BIND, and STRUCT-CAT), and a subset of ciprofloxacin concentrations.

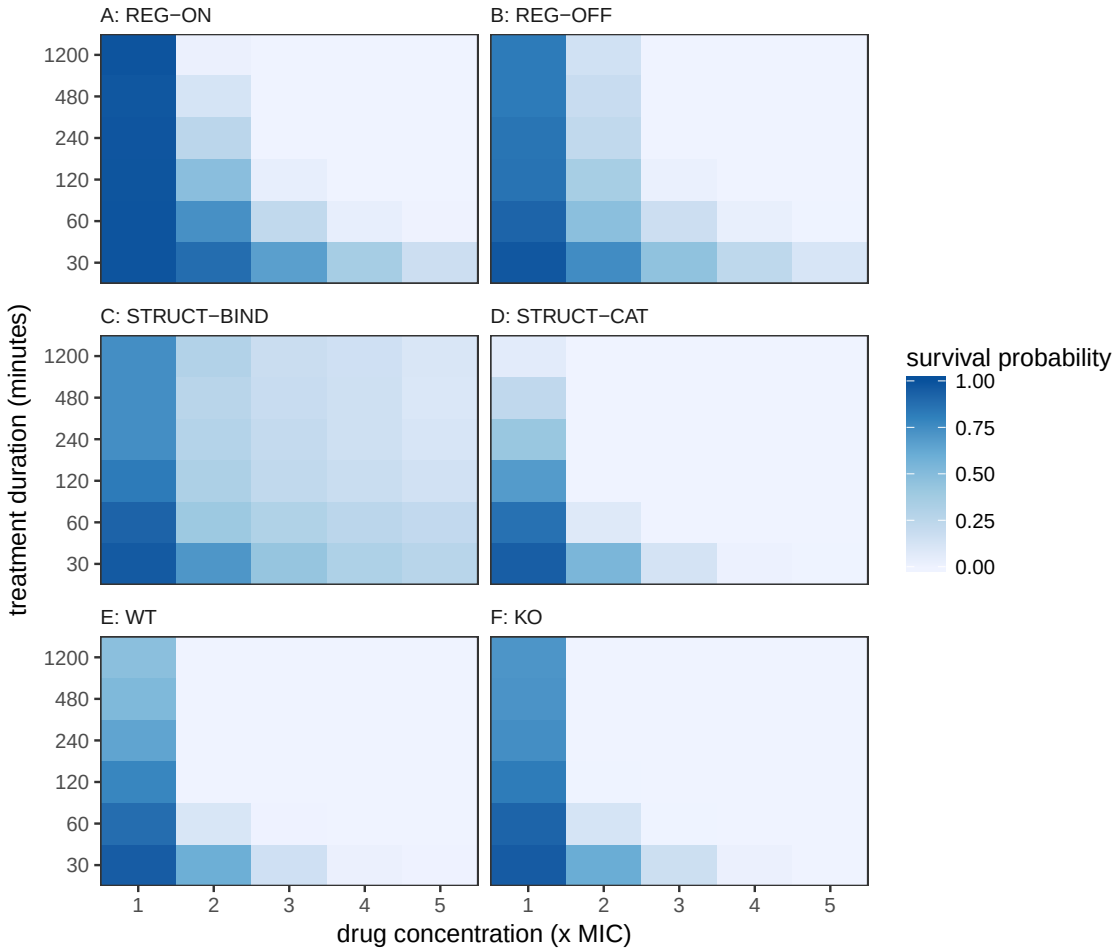

Figure S5: Survival probability after exposure to a pulse of antibiotics. The survival probability is indicated by color scale. The drug dose is applied as a step-function with constant external drug concentration during the pulse, and $c_{\text{out}} = 0$ for the remainder of the experiment up to $t = 1,200$ minutes. Simulations are performed as in Fig. 3 of the manuscript. The mutant effect is $\mu = 200$.

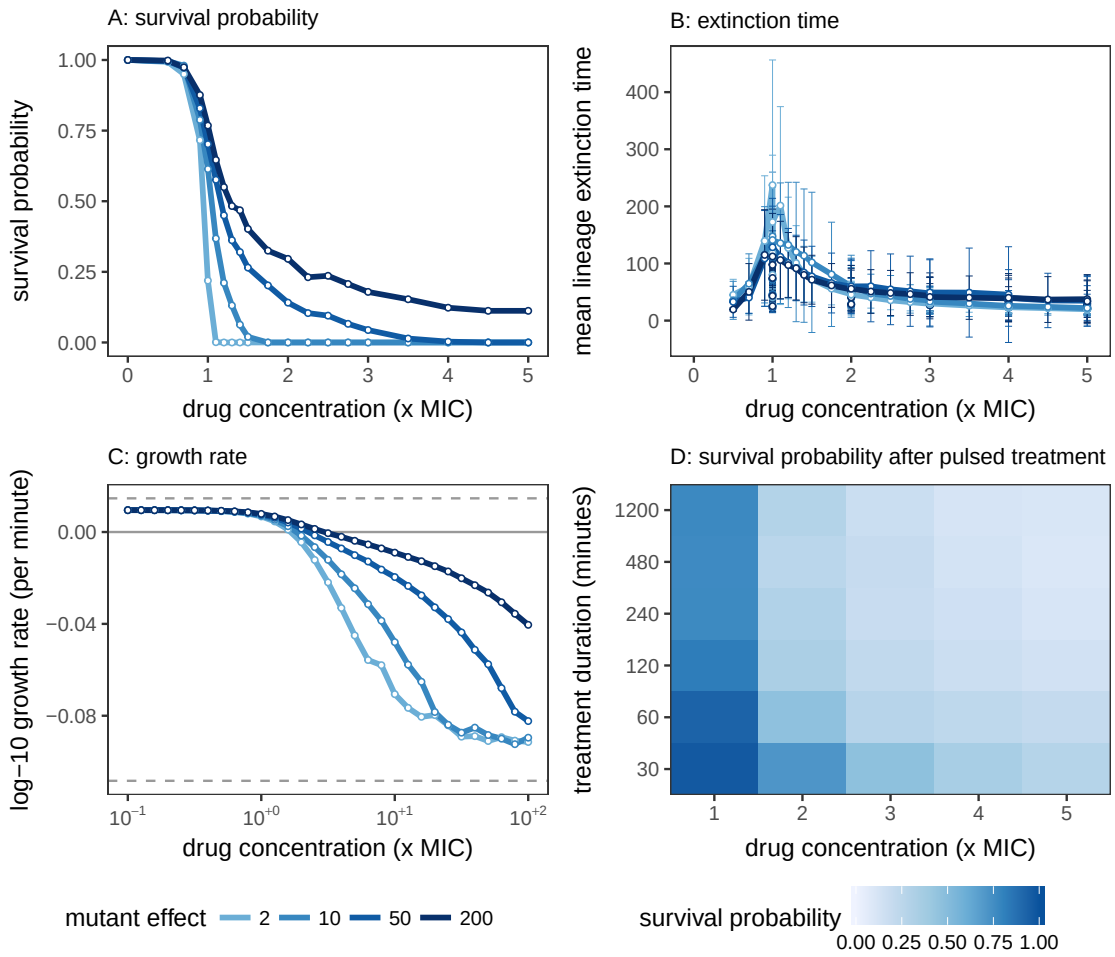

Figure S6: The dynamics of the REG-BURST mutants are very similar to those of STRUCT-BIND. Survival probability (A) and extinction times (B) of the REG-BURST mutants across drug concentrations are calculated as in Fig. 3 of the manuscript and Fig. S3. Color scale indicates the mutant effect (μ). C) The net population growth rates as computed in Fig. 4 of the manuscript. D) Survival probability after exposure to a pulse of antibiotics for the REG-BURST mutant with 200-fold effect. The survival probability is indicated by color scale. Simulations are performed as in Fig. 7 of the manuscript and Fig. S5.

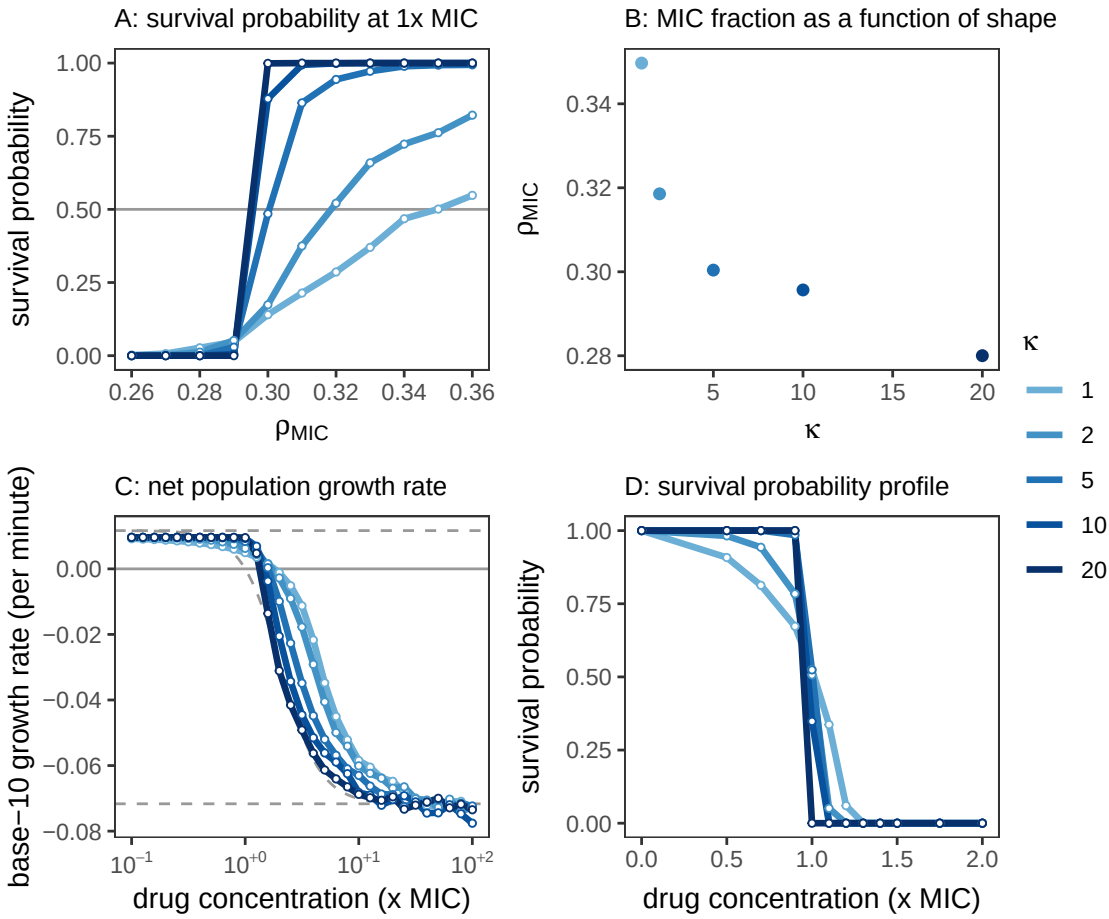

Figure S7: Parameter screening for rifampicin. A) We calculate the survival probability for 1,000 lineages initiated from a single cell at 1× MIC while varying the MIC fraction of bound targets (ρ_{MIC}) and the shape parameter κ. B) From panel A we interpolate the value of ρ_{MIC} which gives a survival probability of 50%. C) For each pair of κ and ρ_{MIC} in panel B, we simulate a population of 10,000 cells under different drug pressure, and extract the net population growth rate. This can be compared with the maximum and minimum reported rates (dashed horizontal lines), and the growth curve reported by Regoes et al. (2004) (dashed curve). D) For each pair of κ and ρ_{MIC} in panel B, we calculate the survival probability of 1,000 lineages initiated from a single cell under different drug pressure.

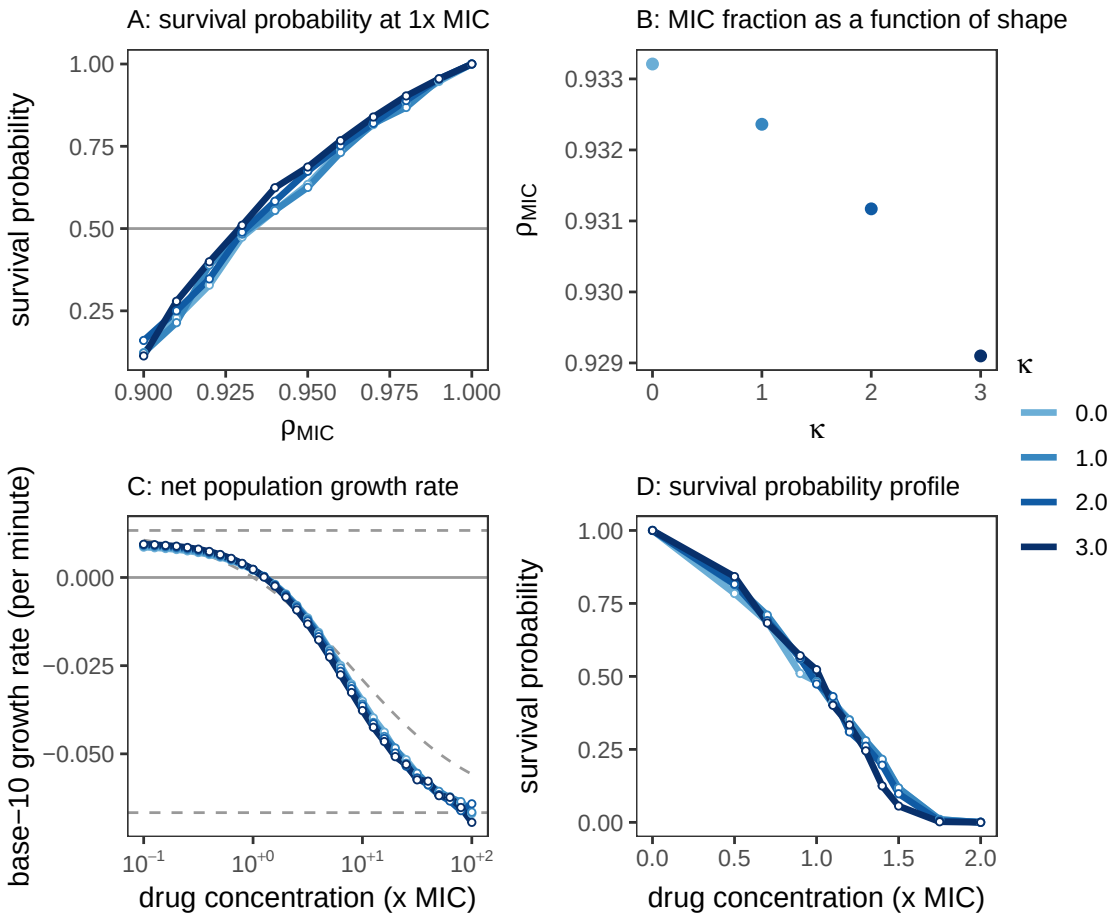

Figure S8: Parameter screening for cefotaxime. A) We calculate the survival probability for 1,000 lineages initiated from a single cell at 1× MIC while varying the MIC fraction of bound targets (ρ_{MIC}) and the shape parameter κ. B) From panel A we interpolate the value of ρ_{MIC} which gives a survival probability of 50%. C) For each pair of κ and ρ_{MIC} in panel B, we simulate a population of 10,000 cells under different drug pressure, and extract the net population growth rate. This can be compared with the maximum and minimum reported rates (dashed horizontal lines), and the growth curve reported by Regoes et al. (2004) for ampicillin (dashed curve). D) For each pair of κ and ρ_{MIC} in panel B, we calculate the survival probability of 1,000 lineages initiated from a single cell under different drug pressure.