SI Appendix

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1. Differential equations and steady-state relations

The ordinary differential equations associated with the model in Fig. 1 of the main text are

$$\begin{aligned}\frac{dr_f}{dt} &= \left(r_0 - r_f\right)k_d - a_f r_f k_a - \mu r_f + \mu r_0 \\ \frac{da_f}{dt} &= a_e k_{in} - a_f k_{out} + \left(r_0 - r_f\right)k_d - a_f r_f k_a - \mu a_f .\end{aligned} \quad [S1]$$

Here, r_0 and r_f are the total and free target concentrations, respectively, while a_e and a_f are the external and free intracellular drug concentrations, respectively, so that the target-bound drug and occupied target concentration, r_b, is r_0 - r_f. The rate constants for drug dissociation from and drug association to the target are k_d and k_a, respectively. μ is the growth rate of the bacterial population, taking the value μ_0 when a_e = 0, while k_{in} and k_{out} are first order rate constants for drug influx from the medium and drug efflux from the cytoplasm, respectively. The latter rate constants are defined in terms of cell membrane area, membrane permeability and pump efficiency as described below for gram positive bacteria with a single membrane and gram negative bacteria with two membranes separated by a periplasmic space (1-3).

Division of both equations with r_0 and μ_0 gives the differential equations

$$\begin{aligned}\frac{1}{\mu_0}\frac{d\tilde{r}_f}{dt} &= \left(1-\tilde{r}_f\right)\left(\tilde{k}_d + \tilde{\mu}\right) - \tilde{k}_a \tilde{a}_f \tilde{r}_f , \\ \frac{1}{\mu_0}\frac{d\tilde{a}_f}{dt} &= \tilde{j}_{in} - \tilde{a}_f\left(\tilde{k}_{out} + \tilde{k}_a \tilde{r}_f + \tilde{\mu}\right) + \left(1-\tilde{r}_f\right)\tilde{k}_d ,\end{aligned} \quad [S2]$$

The dimensionless variables are defined as

$$\begin{aligned}\tilde{r}_f &= \frac{r_f}{r_0} & \tilde{a}_f &= \frac{a_f}{r_0} \\ \tilde{k}_a &= \frac{k_a r_0}{\mu_0} & \tilde{k}_d &= \frac{k_d}{\mu_0} \\ \tilde{k}_{out} &= \frac{k_{out}}{\mu_0} & \tilde{j}_{in} &= \frac{a_e k_{in}}{\mu_0 r_0} \\ \tilde{\mu} &= \frac{\mu}{\mu_0}.\end{aligned} \quad [S3]$$

At the steady-state, Eq. S2 relates the inflow of drug, $\tilde{j}_{in}$, to the growth rate, $\tilde{\mu}$, and the drug-free target concentration, $\tilde{r}_f$, through

$$\tilde{j}_{in} = \left(1-\tilde{r}_f\right)\left(\frac{\left(\tilde{k}_d + \tilde{\mu}\right)\left(\tilde{k}_{out} + \tilde{\mu}\right)}{\tilde{k}_a \tilde{r}_f} + \tilde{\mu}\right), \quad [S4]$$

which is Eq. 2 in the main text. When $\tilde{\mu}$ can be written as a monotonically increasing function, $\tilde{\mu} = \tilde{\mu}(\tilde{r}_f)$, of $\tilde{r}_f$, then the inverse, $\tilde{r}_f = \tilde{r}_f\left(\tilde{\mu}\right)$ is well-defined and can be used in Eq. S4, leading to Eq. 3 in the main text. The special case $\tilde{r}_f = \tilde{\mu}$ leads to Eq. 4 of the main text.

2. Conditions for growth-bi-stability

In this section we specify necessary and sufficient conditions for growth-bi-stability of Eq. 4 in the main text, corresponding to the special case of Eq. S4 (and Eq. 2 in the main text), when $\tilde{r}_f = \tilde{\mu}$. Growth-bi-stability requires two stable and one unstable growth rates, $\tilde{\mu}$, for one $\tilde{j}_{in}$-value (Fig. 2A, main text) in the μ intervals $0 \le \tilde{\mu} \le 1;\ 0 \le \tilde{j}_{in} < \infty$. This is equivalent to $\tilde{j}_{in} = \tilde{j}_{in}\left(\tilde{\mu}\right)$ having one maximum and one minimum in these intervals of $\tilde{\mu}$ and $\tilde{j}_{in}$ (Fig. S2), and this condition defines the necessary and sufficient conditions for growth-bi-stability.

To define the necessary conditions for growth-bi-stability, we note that the maxima and minima of $\tilde{j}_{in}\left(\tilde{\mu}\right)$ are found by solving $\left(d/d\tilde{\mu}\right)\left(\tilde{j}_{in}\left(\tilde{\mu}\right)\right) = 0$, corresponding to

$$\tilde{k}_d\left(\tilde{k}_{out} + \tilde{\mu}^2\right) + \tilde{\mu}^2\left(\tilde{k}_{out} + \left(1+\tilde{k}_a\right)\left(2\tilde{\mu} - 1\right)\right) = 0 \quad [S5]$$

The condition for one maximum and one minimum for $\tilde{j}_{in} = \tilde{j}_{in}\left(\tilde{\mu}\right)$, can be found from the discriminant, *D*, of the cubic equation S5. For discriminant analysis, it is convenient to reduce Eq. S5 from the form $a\tilde{\mu}^3 + b\tilde{\mu}^2 + c = 0$, where

$$\begin{aligned}a &= 2\left(1+\tilde{k}_a\right) \\ b &= \tilde{k}_d + \tilde{k}_{out} - \left(1+\tilde{k}_a\right), \\ c &= \tilde{k}_d \tilde{k}_{out}\end{aligned} \quad [S6]$$

to the "depressed" cubic form $y^3 + py + q = 0$, by the variable change $\tilde{\mu} = y - b/\left(3a\right)$ (4). *D* can then be written as

$$D = \left(\frac{p}{3}\right)^3 + \left(\frac{q}{2}\right)^2 , \quad [S7]$$

with *p* and *q* are defined as

$$p = -\frac{b^2}{3a^2} \quad q = \frac{2b^3}{27a^3} + \frac{c}{a} . \quad [S8]$$

When *D*>0, *D*=0 or *D*<0 the cubic equation has one real and two complex roots, three real roots of which at least two are equal or three distinct real roots, respectively (4, 5). D<0 is a necessary condition for growth-bi-stability, since one real maximum and one real minimum are required (Fig. S2). Insertion of the definitions of Eq. S8 into Eq. S7 gives

$$D = \frac{c\left(4b^3 + 27a^2 c\right)}{108a^4} . \quad [S9]$$

Since *c* and *a* are larger than zero, we define the for root characterization equivalent discriminant, D', through $D' = 108a^4 D/c$, giving

$$D' = b^3 + 27\left(a^2/4\right)c . \quad [S10]$$

Moving the relations of Eq. S6 into Eq. S10 gives *the necessary condition for* growth-bi-stability, visualized in Fig. 2B of the main text, as

$$D' = \left(\tilde{k}_{out} - \left(1+\tilde{k}_a\right) + \tilde{k}_d\right)^3 + 27\tilde{k}_d\tilde{k}_{out}\left(1+\tilde{k}_a\right)^2 < 0 . \quad [S11]$$

To show that the inequality in Eq. S11 is also a *sufficient* criterion for growth-bi-stability, we need to ensure that there is a maximum and a minimum in the

region $0 \le \tilde{\mu} \le 1;\ 0 \le \tilde{j}_{in} < \infty$, *and* that the third root of Eq. S5 has a negative $\tilde{\mu}$-value (Fig. S2). To prove this, the equality just above Eq. S6 is rewritten as

$$-a\tilde{\mu}^2 - b\tilde{\mu} = c / \tilde{\mu}\,, \qquad \text{[S12]}$$

and the roots of this expression are visualized graphically (Fig. S3). The left and right hand sides of Eq. S12 are visualized in Fig. S3 both for *b<0* and *b>0*. There is one negative and real root for all *b* values. When b<0, there will be two positive and real roots in addition to the real negative root, provided that the inequality in Eq. S11 is fulfilled. The positive roots of Eq. S12 are bounded by the left hand side's intersection with the x-axis, meaning that the smallest possible root is zero, while the larger root is given by

$$\tilde{\mu} = -\frac{b}{a} = -\frac{1}{2}\left(\frac{\tilde{k}_d + \tilde{k}_{out}}{1+\tilde{k}_a} - 1\right), \qquad \text{[S13]}$$

bounded above by 1/2. Accordingly, the two positive real roots appear in the region $0 \le \tilde{\mu} \le 1;\ 0 \le \tilde{j}_{in} < \infty$, meaning that the inequality in Eq. S11 is both a necessary and a sufficient condition for growth-bi-stability.

The case of equilibrium between free and target bound drug in the cytoplasm, as assumed by Elf et al. (6), is obtained by dividing both sides of Eq. S11 by $\left(1+\tilde{k}_a\right)^3$ and taking the limit of infinite $\tilde{k}_a$- and $\tilde{k}_d$-values at constant $\tilde{K}_D = \tilde{k}_d / \tilde{k}_a = K_D / r_0$ ratio, which gives

$$\left(-1+\tilde{K}_D\right)^3 + 27\tilde{K}_D\tilde{k}_{out} < 0\,. \qquad \text{[S14]}$$

When $\tilde{K}_D \ll 1$ (see example in section 8, and ref. (6)), this simplifies to

$$\tilde{k}_{out} = \frac{k_{out}}{\mu_0} < \frac{1}{27\tilde{K}_D} = \frac{r_0}{27K_D}\,. \qquad \text{[S15]}$$

3. Growth-rate approximations in the fast and slow growth regimes

In the fast growth regime, the inequality in Eq. 5 holds, so that Eq. 4 can be approximated as

$$\tilde{j}_{in} = \left(1-\tilde{\mu}\right)\tilde{\mu}\,. \qquad \text{[S16]}$$

Solving Eq. S16 for $\tilde{\mu}$, leads to Eq. 6 in the main text.

In the slow growth regime, the inequalities $\tilde{\mu} << 1$, $\tilde{k}_d$, $\tilde{k}_{out}$ hold, so that Eq. 4 can be approximated as

$$\tilde{j}_{in}\tilde{\mu} - \tilde{\mu}^2 = \frac{\left(\tilde{k}_d + \tilde{\mu}\right)\left(\tilde{k}_{out} + \tilde{\mu}\right)}{\tilde{k}_a} = \frac{\tilde{k}_d\tilde{k}_{out}}{\tilde{k}_a}\,. \qquad \text{[S17]}$$

Solving Eq. S17 for $\tilde{\mu}$ leads to

$$\tilde{\mu} = \frac{\tilde{j}_{in}}{2} \pm \sqrt{\frac{1}{4}\tilde{j}_{in}^2 - \frac{\tilde{k}_d\tilde{k}_{out}}{\tilde{k}_a}}\,. \qquad \text{[S18]}$$

Discarding of the unphysical root, leads to Eq. 7 in the main text.

4. Mean time calculations

The mean times to reach steady-state after drug addition (τ_{ss}) and to reach maximal growth rate after drug removal (τ_r) are defined as

$$\tau_{ss} = \int_0^\infty \frac{\tilde{\mu}(t) - \tilde{\mu}_{ss}}{\tilde{\mu}(0) - \tilde{\mu}_{ss}} dt \text{ and } \tau_r = \int_0^\infty \frac{1-\tilde{\mu}(t)}{1-\tilde{\mu}(0)}\, dt\,. \qquad \text{[S19]}$$

Here, μ_{ss} is the steady-state growth rate. The various mean-time areas are visualized in Fig. S7.

5. Robustness of target resistance masking and growth-bi-stability

In the analytical treatment of bi-stability regions and target resistance masking in Fig. 3 of the main text, we assume that $\tilde{r}_f = \tilde{\mu}$. To study the robustness of our qualitative results, we use a general power-law relation between free drug target and growth rate as $\tilde{\mu} = \tilde{r}_f^{\,1/n}$. The cases when *n=1/2* and *n=2* are shown in Fig. S4A and S4B, respectively, illustrating how bi-stability and target resistance masking are robust to variation in the functional relation between $\tilde{r}_f$ and $\tilde{\mu}$, with MIC-values close to 1/2 in all cases. In the slow growth regime**,** the inequalities $\tilde{\mu}, \tilde{r}_f << 1$ and $\tilde{k}_d, \tilde{k}_{out} << \tilde{\mu}$ hold, and Eq. S4 can be approximated by

$$\left(\tilde{j}_{in} - \tilde{\mu}\right)\tilde{\mu}^n = \frac{\tilde{k}_d\tilde{k}_{out}}{\tilde{k}_a}\,. \qquad \text{[S20]}$$

When $\tilde{j}_{in} >> \tilde{\mu}$, then

$$\tilde{\mu} = \left(\frac{\tilde{k}_d\tilde{k}_{out}}{\tilde{k}_a}\frac{1}{\tilde{j}_{in}}\right)^{1/n}\,. \qquad \text{[S21]}$$

Here, $\tilde{\mu}$ is proportional to $1/\tilde{j}_{in}^{1/n}$, meaning that $\tilde{\mu}$ decreases much faster with increasing $\tilde{j}_{in}$ when *n=1/2* (Fig. S4A) than when *n=1* (Fig. 3 of the main text). When *n=2*, the decrease in $\tilde{\mu}$ with increasing $\tilde{j}_{in}$ (Fig. S4B) is, in contrast, much slower than when *n=1*.

6. Dynamics of growth inhibition

The time to establish steady state growth for bacteria after addition or removal of antibiotic drugs can be quite long, in particular near one of the transition points between the fast and slow growth regimes of strains displaying growth-bi-stability (6). Therefore, realistic descriptions of experiments often require that one accounts for the transient growth behavior of drug-exposed bacterial strains. Such experiments can be used to identify or reject the existence of target resistance masking and growth-bi-stability. In the cases with resistance masking and growth-bi-stability there would, for certain drug concentrations, exist two regimes in the dynamics; one where the growth-rate is slowly decreasing in the fast growth regime, and one where the growth rate drops dramatically down to small values (see below).

We first compare the approach to steady state of the drug-efflux deficient strains in Fig. 3 in the main text, when they initially grow in the absence of antibiotics ($\tilde{\mu} = 1$) and then are rapidly exposed to a normalized drug concentration (a_e / r_0) of 0.3, just above their common steady state $MIC_{50\%}$-value of 0.25 (Fig. S5A). The growth rates of all three strains decrease similarly and slowly in the fast growth regime, until their growth rates at the same point in time drop sharply to their different

values in the slow growth regime according to Eq. 8, in the main text. The average times to reach steady state (Eq S19) are virtually identical for the wild type and resistance mutated strains (Fig. S6A). Next, we inspect what happens with the same drug-efflux deficient strains, initially growing in the steady state exposed to a normalized drug concentration of 0.3, after a sudden drug removal from the medium (Fig. S6B). First, the growth rates increase slowly (note the logarithmic time axis), and then they increase more rapidly. The average recovery time after drug removal (S19) is inversely related to the target affinity to the drug (Fig. S6C), *i.e.* the drug resistant strains recover much faster than wild type after drug removal.

Next, we compare the approach to the steady state of the drug-efflux proficient strains in Fig. 3 in the main text, when they first grow in the absence of antibiotics and then are exposed to a normalized drug concentration of 30 000 (Fig. S5C). Here, the growth rate decrease is initially much more rapid than in the drug efflux deficient case. All strains move along the same straight line with finite slope, and then they stop at growth rates inversely proportional to their drug-target affinities. In this case, the average rates to reach steady state increase with increasing drug-target affinity. In the inverse scenario (Fig. S5E), recovery to the maximum growth rate after drug removal from the medium is very much faster than in the drug-efflux deficient case (compare Fig. S5B and S5D), and the drug target-mutant strains have much smaller average recovery time than wild type (Fig. S6D).

7. Detailed definitions of rate constants for transport over the cell envelope

Ordinary differential equations associated with the model in Fig. 1 of the main text, in which the membrane permeability and per surface area pump efficiency are explicit, can in the gram positive case be written as

$$\begin{aligned}\frac{da_f}{dt} &= c_1\left(a_{ext}-a_f\right)-c_{pump}a_f-k_a a_f r_f+k_d a_f-\mu a_f\\ \frac{dr_f}{dt} &= -k_a a_f r_f+k_d\left(r_0-r_f\right)-\mu r_f+\mu r_0.\end{aligned} \quad [S22]$$

Here $c_1=\left(A_1C_1\right)/V_c$ and $c_{pump}=\left(A_1C_{pump}\right)/V_c$, where A_1, C_1, and V_c are the cell membrane area, cell wall permeability and cytoplasmic volume, respectively, and C_{pump} is the drug efflux pump efficiency per surface area. Comparison of Eq. S22 and Eq. S1 defines the first order influx and outflux rate constants k_{in} and k_{out} as

$$\begin{aligned}k_{in} &= c_1\\ k_{out} &= c_1+c_{pump}.\end{aligned} \quad [S23]$$

For gram negative bacteria, where there are two membranes in the cell envelope separated by a periplasmic space, we will consider two separate cases. In the first, the drug efflux pumps are transporting drug molecules from the periplasm to the external medium (1, 2), while in the second case the drug molecules are pumped directly from the cytoplasm to the external medium (7). In the first case, the ordinary differential equations are

$$\begin{aligned}\frac{da_p}{dt} &= \frac{V_c}{V_p}c_1\left(a_{ext}-a_p\right)+\frac{V_c}{V_p}c_2\left(a_f-a_p\right)-\frac{V_c}{V_p}c_{pump}a_p-\mu a_p\\ \frac{da_f}{dt} &= c_2\left(a_p-a_f\right)-k_a a_f r_f+k_d a_f-\mu a_f\\ \frac{dr_f}{dt} &= -k_a a_f r_f+k_d\left(r_0-r_f\right)-\mu r_f+\mu r_0.\end{aligned} \quad [S24]$$

Here, $c_1=\left(A_1C_1\right)/V_c$, $c_2=\left(A_2C_2\right)/V_c$ and $c_{pump}=\left(A_1C_{pump}\right)/V_c$, where, C_1, A_1, C_2, A_2, and C_{pump} are the outer membrane permeability, the outer membrane area, the inner membrane permeability, the inner membrane area and the per inner surface area pump efficiency, respectively. Furthermore, a_p and V_p are the periplasmic drug concentration and the periplasmic volume, respectively. At the steady-state, a_p is given by

$$a_p=\frac{\frac{V_c}{V_p}\left(c_1a_{ext}+c_2a_f\right)}{\frac{V_c}{V_p}\left(c_1+c_2+c_{pump}+\mu\frac{V_p}{V_c}\right)}\approx\frac{c_1a_{ext}+c_2a_f}{c_1+c_2+c_{pump}}. \quad [S25]$$

The approximation to the right in Eq. S25, valid when the term $\mu V_p/V_c$ is relatively small, defines the first order influx and outflux rate constants k_{in} and k_{out} as

$$\begin{aligned}k_{in} &= c_2\frac{c_1}{c_1+c_{pump}+c_2}\\ k_{out} &= c_2\frac{c_1+c_{pump}}{c_1+c_{pump}+c_2}.\end{aligned} \quad [S26]$$

When the term c_2 dominates in the denominators of Eq. S26, increasing pump efficiency increases k_{out} in proportion to c_1+c_{pump} with k_{in} unaltered. As c_{pump} increases further, k_{out} will reach a plateau while k_{in} will decrease (Fig. S1, blue curves).

In the second case of gram negative bacteria, where the drug efflux pumps are assumed to transport drug molecules directly from the cytoplasm into the external medium, the differential equations for the model in Fig 1 in the main text are given by

$$\begin{aligned}\frac{da_p}{dt} &= \frac{V_c}{V_p}c_1\left(a_{ext}-a_p\right)+\frac{V_c}{V_p}c_2\left(a_f-a_p\right)-\mu a_p\\ \frac{da_f}{dt} &= c_2\left(a_p-a_f\right)-c_{pump}a_f-k_a a_f r_f+k_d a_f-\mu a_f\\ \frac{dr_f}{dt} &= -k_a a_f r_f+k_d\left(r_0-r_f\right)-\mu r_f+\mu r_0.\end{aligned} \quad [S27]$$

Here, $c_{pump}=A_2C_{pump}/V_c$. The steady state concentration of drug molecules in the periplasm is now given by

$$a_p=\frac{c_1a_{ext}+c_2a_f}{c_1+c_2+\mu\frac{V_p}{V_c}}\approx\frac{c_1a_{ext}+c_2a_f}{c_1+c_2}. \quad [S28]$$

The steady state rate constants k_{in} and k_{out} are here defined as

$$k_{in} = c_2 \frac{c_1}{c_1 + c_2}$$
$$k_{out} = c_2 \left(\frac{c_1}{c_1 + c_2} + \frac{c_{pump}}{c_2} \right). \quad [S29]$$

In this case, k_{in} is independent of the drug efflux pump efficiency, and k_{out} increases linearly with increasing pump efficiency (Fig. S1, red curves).

The usefulness of the steady state rate constants k_{in} and k_{out} also under pre-steady-state was tested by re-running the simulations in Fig. 4 in the main text using the detailed models of Eq. S24 and Eq. S27, with the following parameter choices:

$$\frac{c_1}{\mu_0} = 1 \qquad \frac{V_c}{V_p} = 30$$
$$\frac{c_{pump}}{\mu_0} = 10 \quad \frac{a_e}{r_0} = 0.3 \quad \text{(efflux pump deficient)} \quad [S30]$$
$$\frac{c_{pump}}{\mu_0} = 10^6 \quad \frac{a_e}{r_0} = 10^3 \quad \text{(efflux pump proficient)}$$

In the first case, the inner membrane flux needs to be fast enough to allow for the very efficient efflux pump rate used in Fig. 4 B and D, and therefore $c_2 / \mu_0 = 10^8$ was used. In the second case, $c_2 / \mu_0 = 10^4$ was used. In both cases, the reduced model in the main text approximates the more detailed models with two cell membranes and a periplasmic space in between.

8. Target resistance masking illustrated by a simple example

One may ask if the condition for target resistance masking and growth-bi-stability is too stringent to be relevant for "real" bacterial pathogens. To inspect this, we discuss a simple example, based on the condition for growth-bi-stability and target resistance masking when free and target bound drugs are equilibrated (See Figure 1, ref. (6), and Eq. S15):

$$k_{out} < \frac{r_0 \cdot \mu_0}{27 \cdot K_D}. \quad [S31]$$

We assume that the dissociation constant, K_D, for drug binding to target is 1 nM for wild type and 10 nM for mutant, the drug free growth rate μ_0 is $2 \cdot 10^{-4}$ s^{-1} corresponding to a generation time of about one hour, the drug target concentration r_0 is 20 μM, so that the inequality in Eq. S31 becomes $k_{out} \leq 0.15$ s^{-1} for target wild type and $k_{out} \leq 0.015$ s^{-1} for target mutant. We also assume that, in the absence of drug efflux pump activity, the outflow rate constant, k_{out}, is 0.01 s^{-1}, corresponding to an effective membrane permeability of $1.7 \cdot 10^{-7}$ cms^{-1} with A/V $= 5.9 \cdot 10^4$ cm^{-1} (8). This permeability is lower than estimates, based on a model system, of the permeability for diffusion of tetracycline over the *E. coli inner* membrane, of $6 \cdot 10^{-6}$ cms^{-1} (9), $3 \cdot 10^{-6}$ cms^{-1} (10) but higher than an estimate of $2 \cdot 10^{-9}$ cms^{-1} based on experiments with *E. coli* cells (8). We define the efflux pump deficient condition as k_{out} = 0.01 s^{-1}, intermediate pump activity as k_{out} = 0.15 s^{-1} and drug efflux pump proficient condition as k_{out} = 2 s^{-1}. The inequality Eq. S31 is then satisfied for both mutant and wild type under efflux pump deficient condition, for wild type only under intermediate pump activity condition and for neither wild type nor mutant under pump efflux proficient condition, implying complete target resistance masking, partial masking or virtually no masking, respectively (Fig. S8). This invented example, showing target resistance masking for realistic values of membrane permeability and target affinity, illustrates the deep connection between growth-bi-stability (the inequality in Eq. S31) and target resistance masking (Fig. S8, Eq. 5 in main text). Indeed, the inequality in Eq. S31 approximates the inequality in Eq. 5 in the limit of equilibration between target bound and free drug in the cytoplasm if "much smaller than" (<<) in Eq. 5 is interpreted as <1/27.

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