Supporting Information

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SI Text

Model Background. Some of the earliest identified toxin–antitoxin systems were *ccdAB* (1), *kis-kid* (2), *pemIK* (identical to *kis-kid*) (3), and *phd-doc* (4), found on plasmids F, R1, R100, and P1, respectively. In those systems, the toxin–antitoxin locus appears to act as a so-called addiction module, ensuring the stable maintenance of the plasmid. Following plasmid loss, the toxin outlasts the labile antitoxin and kills the cell, albeit in a variety of manners: *ccdAB* blocks DNA replication via DNA gyrase (5, 6), whereas *kis-kid* and *phd-doc* target different aspects of the translational machinery (7, 8). Eventually, toxin–antitoxin systems were identified in chromosomes as well. The *mazEF* locus, originally dubbed *chpA*, or chromosomal homologous to *pem* (9), was followed by others, including *relBE* (10) and *yefM-yoeB* (11). In fact, the distinction between plasmid-borne and chromosomal toxin–antitoxin systems is blurry: *ccdAB*, originally found on plasmid F, can also be found on the chromosome of *Escherichia coli* O157 (12). On the basis of the form of the antitoxin and its method of toxin neutralization, toxin–antitoxin systems are classified as type I, II, or III. Type II systems, which we model in this work, account for more than half the toxin–antitoxin systems in *E. coli* and are characterized by an antitoxic protein, as opposed to RNA, that binds directly to the toxic protein (13). Like their plasmid-borne counterparts, chromosomal toxin–antitoxin systems were first described as suicide modules, the executors of programmed cell death (14). However, it was later shown that poisoned cells could recover, suggesting that chromosomal toxin–antitoxin systems instead induce reversible bacteriostasis (15), reminiscent of a persistent cell.

Known Molecular Mechanisms. Toxin and antitoxin syntheses are translationally coupled and the relative positioning of genes ensures more antitoxin is produced (4, 16, 17). The antitoxin neutralizes the toxin by forming a tight complex (14, 18–22) and can bind more than one toxin (23–27). In fact, the ability to bind a second toxin has probably led to at least one debate regarding toxin–antitoxin stoichiometries in solution (23, 27, 28). The antitoxin is also an autorepressor that binds two or more operators. In most well-studied toxin–antitoxin systems, the operators are dissimilar, with one dominant, high-affinity site (25, 28–32). Furthermore, the antitoxin increases in both affinity and cooperativity when in complex with a toxin (25, 28, 30, 32–34), in at least some cases via a bridging mechanism (31, 35–37). However, when the antitoxin binds a second toxin, its affinity and cooperativity are reduced—this conditional cooperativity controls the ratio of toxin to antitoxin, in addition to the overall concentrations. Increased proteolytic activity removes the relatively labile antitoxin (14, 38–42), allowing the free toxin to inhibit cellular translation in various ways (43). Details vary: the number of operator sites, the target of translational inhibition, and whether the stable toxin is monomeric or dimeric. Nevertheless, the picture remains remarkably consistent across a broad spectrum of systems.

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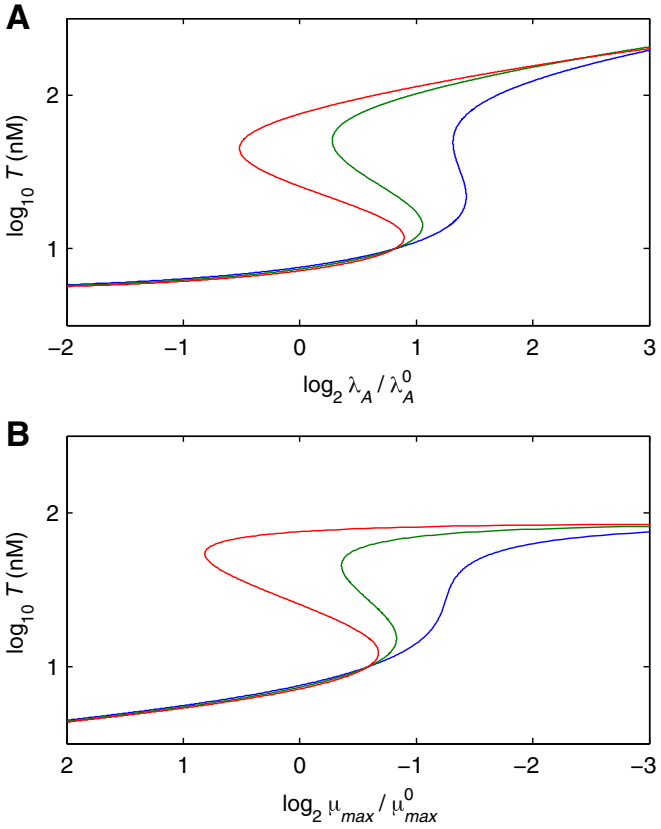

Fig. S1. Varying the cooperativity of toxic inhibition. Steady-state toxin concentrations for $n = 2$ (blue), 2.5 (green), and 3 (red) are shown. (*A*) Varying λ_A while holding $\mu_{max}/\mu_{max}^0 = 1$. (*B*) Varying μ_{max} while holding $\lambda_A/\lambda_A^0 = 1$. As n increases, the width of the bistable buffer increases.

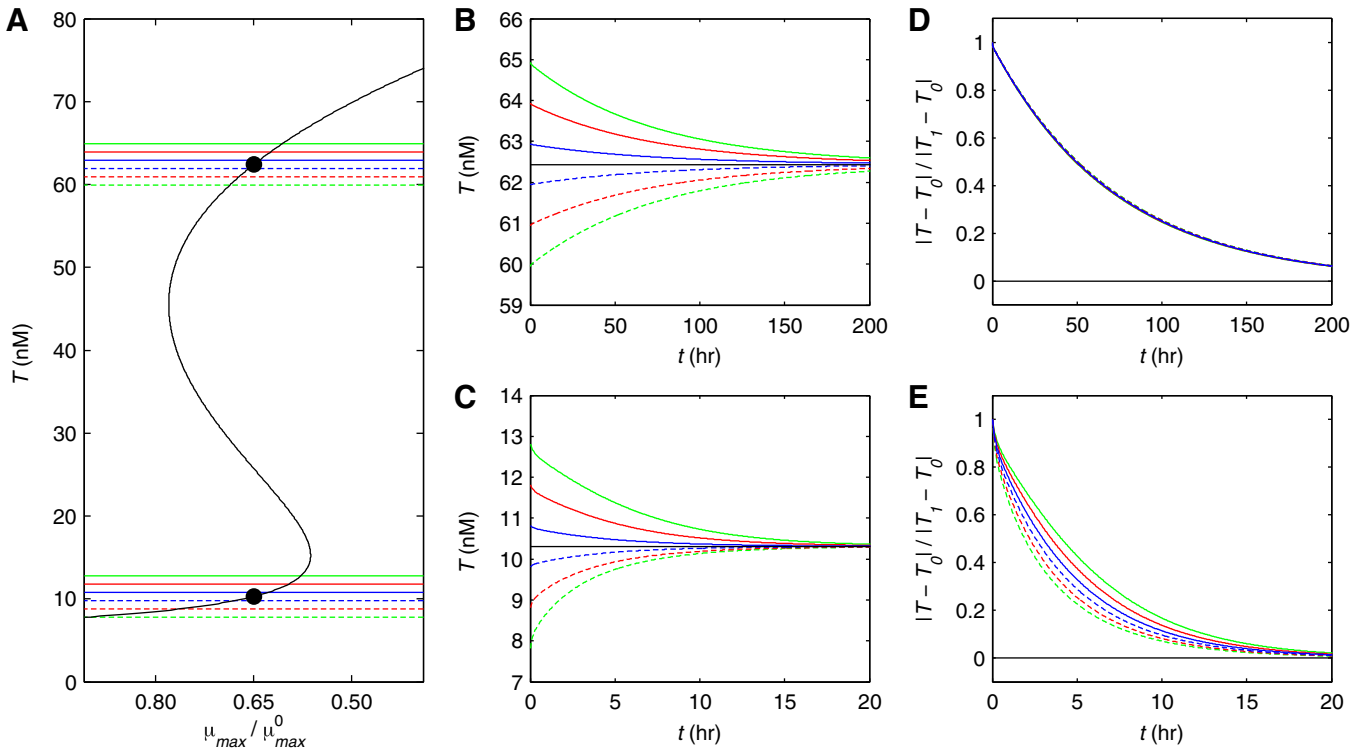

Fig. S2. Dynamic response to small perturbations in toxin concentration. (*A*) For $n = 2.5$ and $\mu_{max}/\mu^0_{max} = 0.65$, without noise, the steady-state toxin concentration (black circle) is perturbed by +0.5 nM (solid blue line), +1.5 nM (solid red line), +2.5 nM (solid green line), −0.5 nM (dashed blue line), −1.5 nM (dashed red line), or −2.5 nM (dashed green line) and then allowed to return to steady state. (*B* and *C*) Dynamic responses to the instantaneous change starting from either the high (*B*) or the low (*C*) toxin concentrations. (*D* and *E*) The dynamic responses shown in *B* and *C*, respectively, normalized to initial (T_1) and final (T_0) steady-state values.

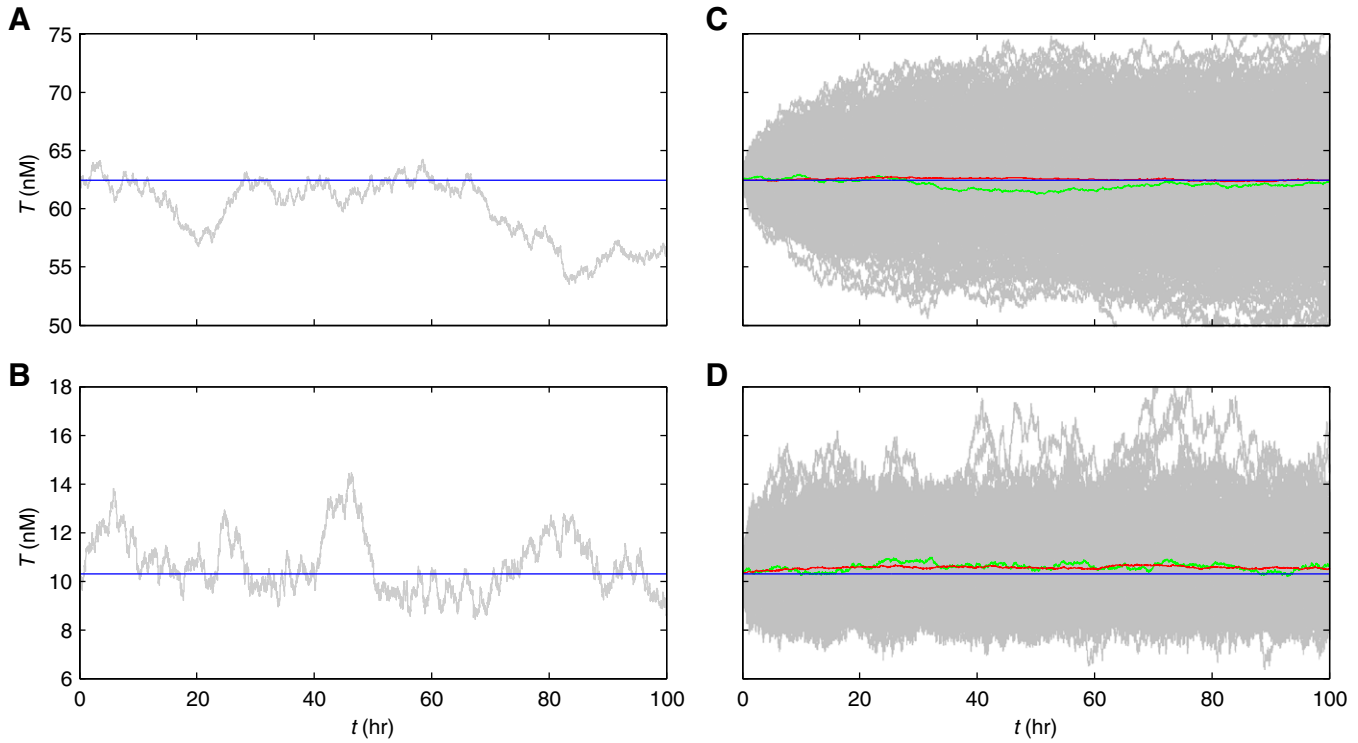

Fig. S3. Stochastic simulation of a single system with small perturbations. (*A* and *B*) For $n = 2.5$ and $\mu_{max}/\mu^0_{max} = 0.65$, the system was poised at either the high (*A*) or the low (*B*) steady-state toxin concentration and then simulated with stochastic noise ($d = 0.1$) (gray) or without noise (blue). (*C* and *D*) Starting from the same high (*C*) or low (*D*) toxin concentrations, the tests were repeated 500 times (gray). The mean of 50 of the stochastic simulations (green) and the mean of all 500 stochastic simulations (red) are compared with the deterministic simulation without noise (blue).

Table S1. Parameter measurements and estimates

Parameter	*kis-kid*	*ccdAB*	*mazEF*	*phd-doc*	*relBE*	*yefM-yoeB*	Used
p	2 (1, 2)	3 (3)	3 (4)	2 (5)	2 (6)	2 (7)	2
n							2
σ	2 (8)			14 (9)	10 (10)		10
α, nM/min							1
t_μ, min							30
t_A, min		30 (11, 12)	30 (13)	120 (14)	>60 (10)	60 (15)	60
t_T, h		>2 (11)	>4 (13)				48
K_H, nM		10–10,000 (16) 0.020 (17)*	<100 (18)*	350 (19) 240 (19)*,†	0.33 (10) 154 (20)*	400 (15)*	100
K_{P1}, μM	2.4–4.8 (2)‡	2.8–3.6 (21)‡ 1.6–2.4 (16)‡ 2.5 (3)	1–4 (22)‡ 2.5–2.8 (23)	0.2 (24) 0.24 (25) 0.25–0.5 (19)‡	4.1–16.6 (6)‡ 12.5 (26)	>0.25 (7)‡	1
K_{P2}, nM		<400 (21)‡,§ <440 (16)‡,§	<100 (22)‡,§	20 (24) <50 (19)‡,§ 300 (19)†	3.4 (6) 12.5–75 (6)‡ 23.4 (26)	<12 (7)‡ 200–800 (27)‡	10
K_{T1}, nM	<500 (28)‡,¶				<0.0011 (29)‡,¶		10
K_{T2}, nM							100

Values are listed by toxin–antitoxin system, including experimental values culled from published literature and estimates based on published figures. Values used in our model are listed in the last column. For simplicity, we list the normal cellular doubling time, t_μ, and the normal half-lives of the toxin and antitoxin, t_T and t_A, rather than the corresponding rate constants μ^0_{max}, λ^0_T, and λ^0_A, which are trivially related by $\mu^0_{max} = \ln 2/t_\mu$, $\lambda^0_T = \ln 2/t_T$, and $\lambda^0_A = \ln 2/t_A$.

*Experiments used a monomeric antitoxin fragment.

†Values appear in the cited article's supplemental information.

‡Ranges are estimates based on published EMSAs.

§Estimates assume all antitoxin is in complex with toxin.

¶Experiments measured translation of single proteins in vitro.

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