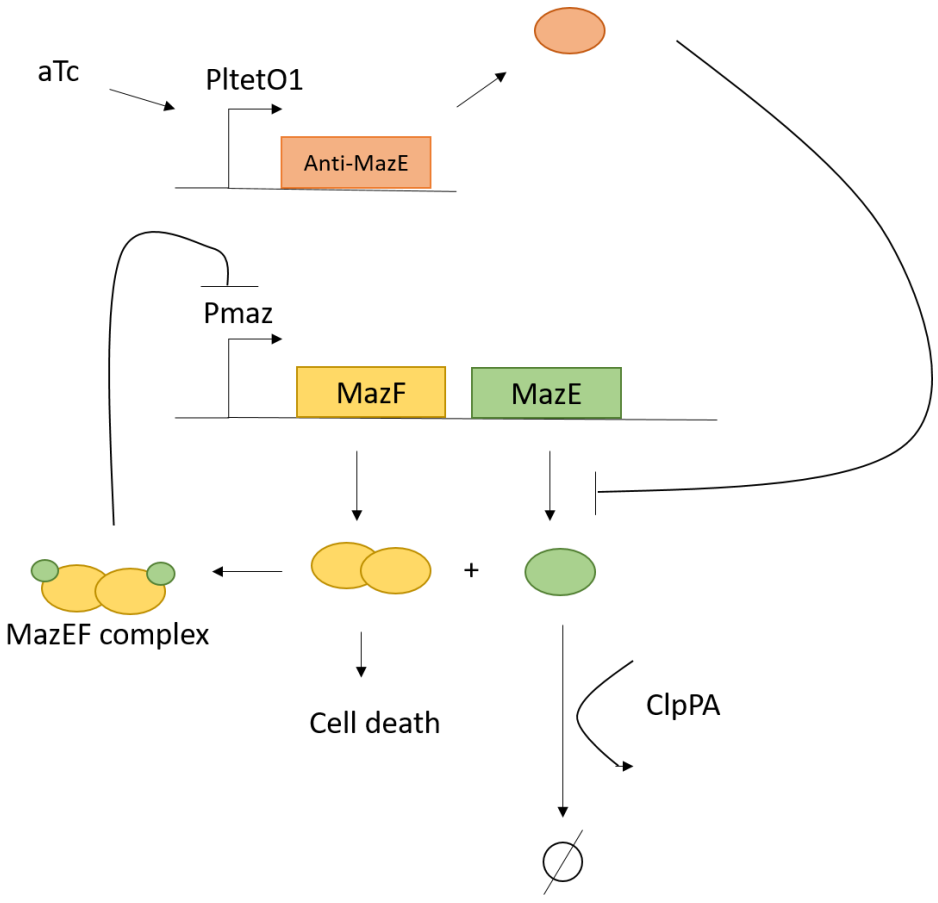
Collaboration with the Freiburg iGem team 2016
Kill switch based on the MazEF system

Short description:

The MazEF system is found in *E.Coli* and *Bacillus Subtilis*. The system consists in two adjacent genes *mazE* and *mazF* located downstream the *relA* gene.

MazE and MazF are co-transcribed under a Pmaz promoter.

MazF is a toxin while MazE is the anti-toxin. The MazEF complex also inhibits the Pmaz promoter. The idea of the switch is to synthesize under certain conditions the anti-MazE protein which prevents translation of the MazE protein. Thus it will prevent it from binding MazF. The concentration of MazF in the circuit will increase and lead to cell death.



MODEL 1

The system is described by the following set of reactions:

aTc-->antiMazE

Pmaz-->mRNA_MazE

mRNA_MazE-->

Pmaz-->mRNA_MazF

mRNA_MazF-->

antiMazE+MazE-->

antiMazE -->

mRNA_MazF -->MazF

MazF-->

mRNA_MazE -->MazE

MazE+ClpAP-->ClpAP

MazE-->

*2*MazF<-->DmazF*

DMazF+MazE<-->DMazF_E

DMazF_E+MazE<-->DMazF_E2

*3*DMazF_E2+Pmaz<-->Pblock*

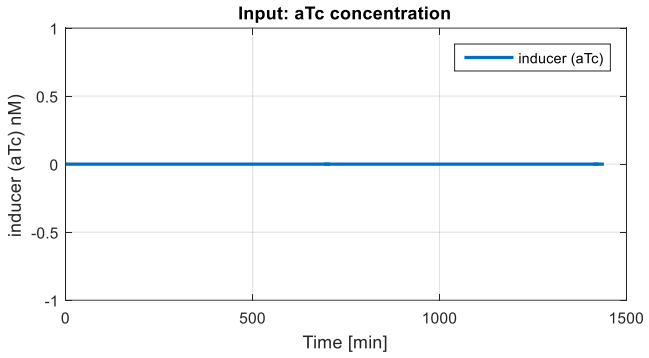
All the equation were derived from this set using mass action kinetic, using the following set of parameters:

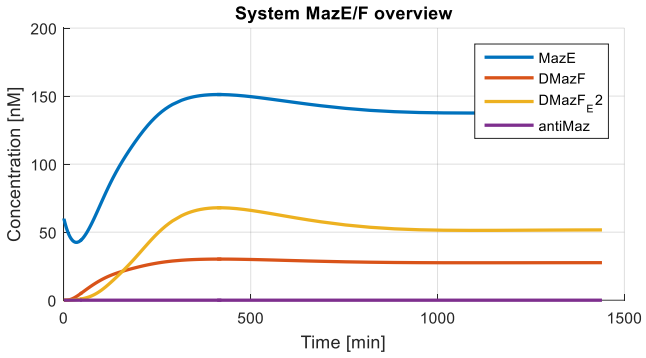
Name	Value	Unit	Source	Description
kProd	150	nM-1min-1	Inferred from [3] and [6]	constitutive production of MazE/F
dmrnaMazE	0.1	min-1		
dmrnaMazF	0.1	min-1		
dmazE	0,1	min-1	Standard degradation rate [8]	degradation of MazE
dmazF	0,1	min-1	Standard degradation rate[8]	degradation of MazF
dantiMaz	0,1	min-1	Standard degradation rate[8]	degradation of antimaz
kdmazf	0,1	nM-1min-1	Inferred from[5,7] based on Kd values	dimerizatioin rate of MazF
k_dmazf	1	min-1	Inferred from[5,7] based on Kd values	dissociation rate of mazf
kdmazfe	0,01	nM-1min-1	Inferred from[5,7] based on Kd values	mazE-MazF binding rate
k_dmazfe	1	Min-1	Inferred from[5,7] based on Kd values	mazE-MazF unbinding rate
kdmazfe2	0,1	nM-1min-1	[Inferred from[5,7] based on Kd values	mazF_E - mazE binding
k_dmazfe2	10	Min-1	Inferred from[5,7] based on Kd values	mazF_E - mazE unbinding
kpbloc	0,0001	nM-1min-1	Inferred from[5,7] based on Kd values	MazF_E2 binding to DNA strand
k_pbloc	2	Min-1	Inferred from[5,7] based on Kd values	MazF_E2 unbinding from DNA strand
cfucoeff	14400	s	Inferred from[1]	Time constant of the cfu in the Maz system
Kanti	0,02	nM	Obtained by fitting dose response curve from [5]	Michaelis-Menten kinetic constant
nanti	1	none	Obtained by fitting dose response curve from [5]	Michaelis-Menten kinetic coeff
Kinhib	0,02	nM	Invented based on standard values	Michaelis-Menten kinetic constant
ninhib	1	none	Chosen because only 1 antiMazE is needed	Michaelis-Menten kinetic coeff
kl	0,1	min-1	[5]	leakiness
ClpAP	10	nM	[2]	Internal concentration of ClpAP
kantimaz	3	nM-1min-1	Inferred from [1]	Antimaz Promoter activity
ka	5	m/mRNA-1min-1	Standard [6]	Transcription rate

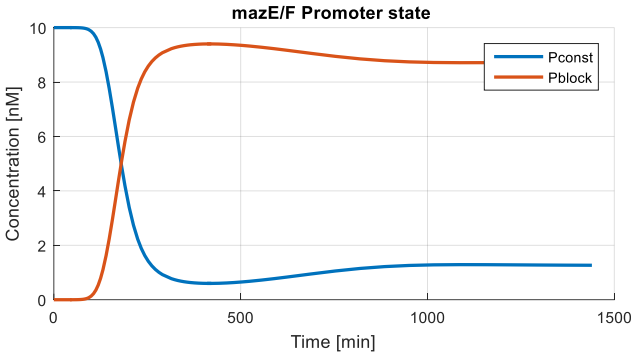
Promoter strength (for antiMazE) and the colony-forming unit (*cfu*) effect on MazF accumulation in cells have been deduced from the paper [1] and [3].
To estimate species concentration from relative light unit (*rlu*) units, we use data conversion found on promega using the methods described on the papers.
This allow us to estimate a steady state concentration of around 28 nM of antiMazE mRNA under 300 ng/L of aTc induction for PLtetO1.

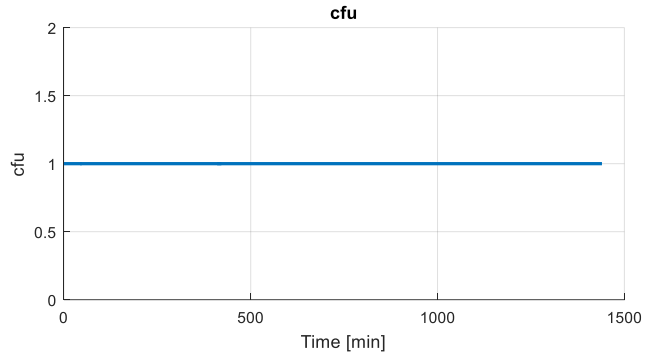
Bionumbers [6] gives us a translation rate in Bacillus of around 5 protein per min per mRNA molecule.
Paper [3] tells us that with around 0.1uM of MazF, cell death appears after about 4h.

Experiment 1 : no aTc









experiment 2: atc is added

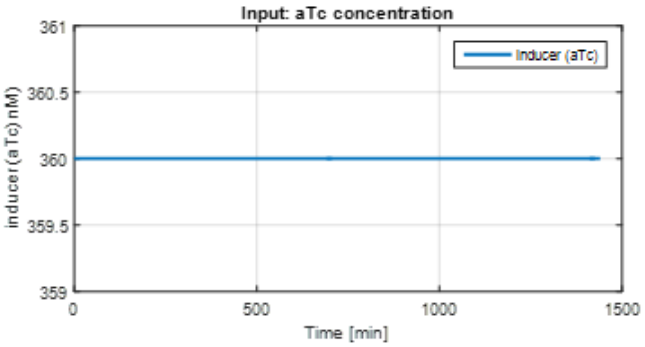
Conclusion:

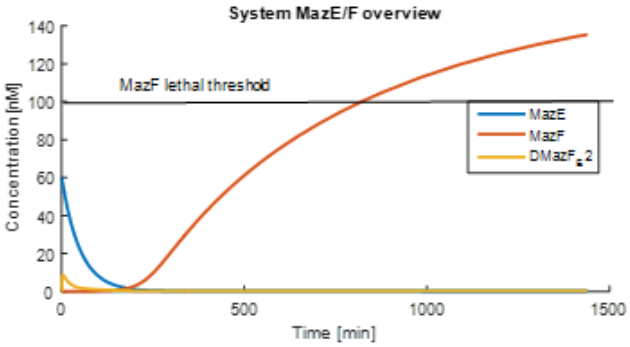
As we can see, a concentration of 300 nM of aTc is enough to induce cell death via the described MazE/F system. aTc is a toxin, but such a low concentration remains inoffensive to cell. Thus the above mechanism can be used as an effective kill-switch for both in vivo and in vitro experiments, without threatening the other microbiota.

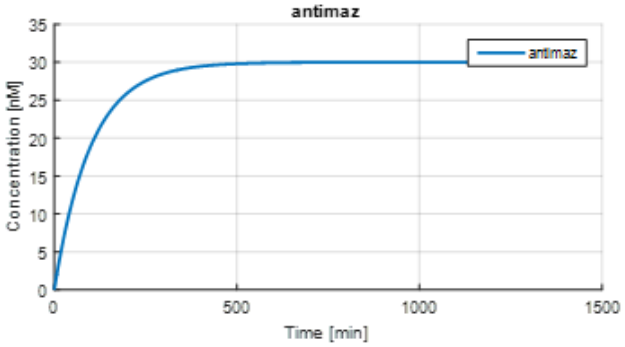
MODEL 2:

A simpler approach consists in modelling all the main interactions with Hill and Michaelis.

The following set of equation was used:







$$\frac{dantimaZ}{dt} = \alpha_1 k_{anti} + \frac{k_{anti}(\frac{aTc}{K_{anti}})^{n_{anti}}}{1 + (\frac{aTc}{K_{anti}})^{n_{anti}}} - D_{anti} antimaZ$$

$$\frac{dMazE}{dt} = \alpha_{maz} k_{prod} + \frac{k_{prod}}{(1 + \left(\frac{antimaZ}{K_{toxi}}\right)^{n_{toxi}})(1 + \left(\frac{MazE\_F2}{K_{inhib}}\right)^{n_{inhib}})} - D_{mazE} MazE$$

$$\frac{dMazF}{dt} = \alpha_{maz} k_{prod} + \frac{k_{prod}}{(1 + \left(\frac{DMazE\_F2}{K_{inhib}}\right)^{n_{inhib}})} - D_{mazF} MazF$$

$$DMazF = \frac{2k_{dmazF} MazF^2}{k_{-dmazF}}$$

$$DMazE_{F2} = \frac{MazE^2\ DMazF}{Kd1\ Kd2}$$

Using the following set of parameters:

Name	Value	Unit	Source	Description
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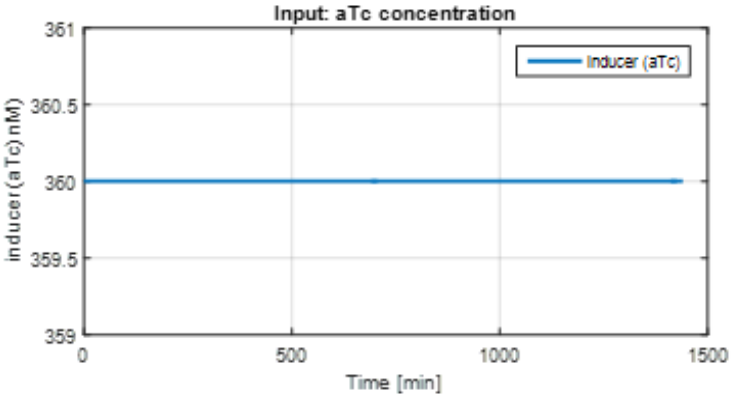
Kanti	0,02	nM	Obtained by fitting dose response curve from [5]	Michaelis-Menten kinetic constant
nanti	1	none	Obtained by fitting dose response curve from [5]	Michaelis-Menten kinetic coeff
Kinhib	0,02	nM	Obtained by fitting dose response curve from [5]	Michaelis-Menten kinetic constant
ninhib	1	none	Chosen because only 1 antiMazE is needed	Michaelis-Menten kinetic coeff

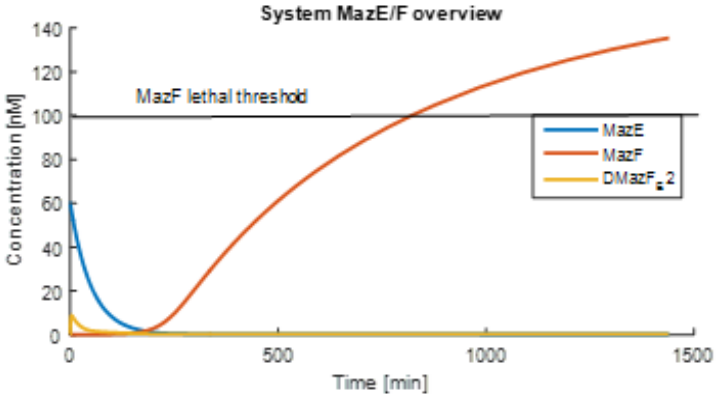
kantimaz	3	nM-1min-1	Inferred from [1]	Antimaz Promoter activity
K_{toxi}	0.1	nM	Invented based on standard values	Hill kinetic constant
n_{toxi}	1	none	Assumed	Hill coeffiscient
α_{maz}	0.1	none	assumed	Basal expression of the mazEF promoter
α_{anti}	0.1	none	assumed	
$Kd1$	100	nM	[5,7]	MazF+MazE<->MazEF affinity
$Kd2$	100	nM	[5,7]	MazEF+MazE<->MazEF2 affinity

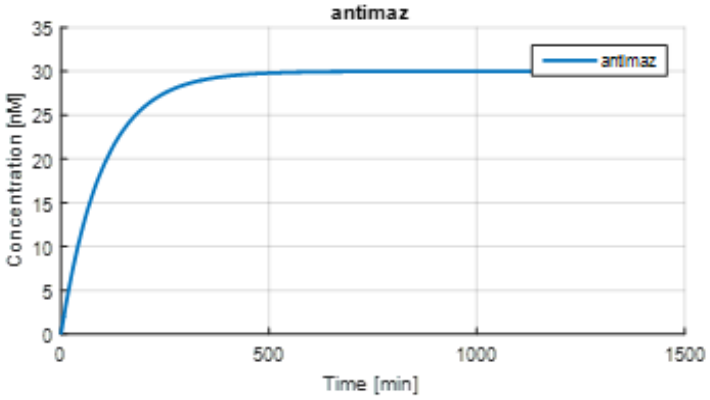
Quasi Steady State approximation has been applied to the following species: DMaZF, DMazE_F.
The colony-forming unit (*cfu*) effect due to MazF accumulation in cells have been deduced from the paper [1] and [3]. It has been assumed that the decrease is both dependant of the time concentration of the toxic compound, and shows an exponential behavior. The curve for *cfu* is made up, based on the observation of paper [1] and [3] for thus concentration of MazF in cells.

Conclusion:

See figure below, the system behave as an effective kill-switch. The amount of aTc necessary is low enough not to trigger cell damage.







References:

[1] Lutz, R. "Independent and Tight Regulation of Transcriptional Units in Escherichia Coli via the LacR/O, the TetR/O and AraC/I1-I2 Regulatory Elements." *Nucleic Acids Research* 25.6 (1997): 1203-210. Web.

[2] Erental, Ariel, Idith Sharon, and Hanna Engelberg-Kulka. "Two Programmed Cell Death Systems in Escherichia Coli: An Apoptotic-Like Death Is Inhibited by the MazEF-Mediated Death Pathway." *PLoS Biology PLoS Biol* 10.3 (2012): n. pag. Web.

[3] Mok, W. W. K., J. O. Park, J. D. Rabinowitz, and M. P. Brynildsen. "RNA Futile Cycling in Model Persisters Derived from MazF Accumulation." *MBio* 6.6 (2015): n. pag. Web.

[4] Rensburg, Julia J. Van, and Paul J. Hergenrother. "Detection of Endogenous MazF Enzymatic Activity in Staphylococcus Aureus." *Analytical Biochemistry* 443.1 (2013): 81-87. Web.

[5] Aizenman, E., H. Engelberg-Kulka, and G. Glaser. "An Escherichia Coli Chromosomal "addiction Module" Regulated by Guanosine [corrected] 3',5'-bispyrophosphate: A Model for Programmed Bacterial Cell Death." *Proceedings of the National Academy of Sciences* 93.12 (1996): 6059-063. Web.

[6] Marianovsky, I., E. Aizenman, H. Engelberg-Kulka, and G. Glaser. "The Regulation of the Escherichia Coli MazEF Promoter Involves an Unusual Alternating Palindrome." Journal of Biological Chemistry 276.8 (2000): 5975-984. Web.

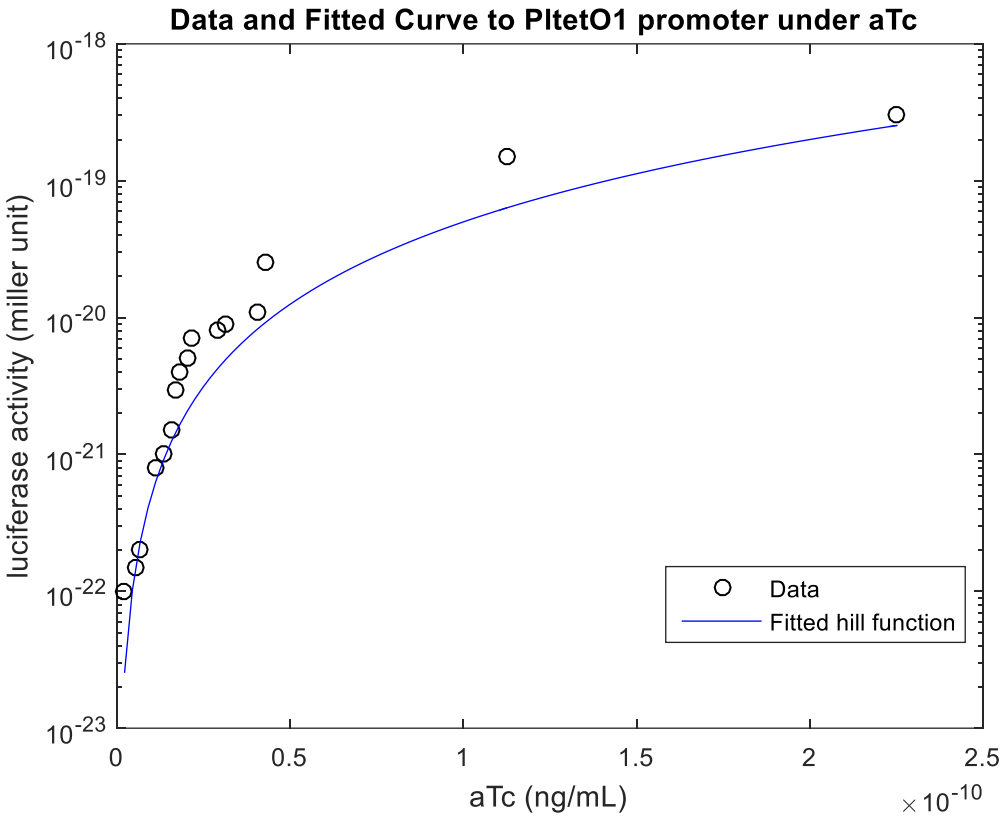
[7] Li, G., Zhang, Y., Chan, M. C., Mal, T. K., Hoeflich, K. P., Inouye, M., & Ikura, M. (2006). Characterization of Dual Substrate Binding Sites in the Homodimeric Structure of Escherichia coli mRNA Interferase MazF. *Journal of Molecular Biology, 357*(1), 139-150. doi:10.1016/j.jmb.2005.12.035

[8] bionumbers.hms.harvard.edu

[9] promega.com

ANNEXES:

Parameters fitting used to determine the strength of the promoter.


Data and Fitted Curve to PltetO1 promoter under aTc
luciferase activity (miller unit)
aTc (ng/mL)
$\times 10^{-10}$
Data
Fitted hill function