Logic gates

Cells receive a wide variety of cellular and environmental signals, which are often processed combinatorially to generate specific genetic responses. The expression of a gene is regulated by other proteins (Transcription Factors). This regulation is due to the interaction between TFs and their binding sites on DNA sequences, which inhibits or promotes the binding of RNApol to the gene to start transcription.

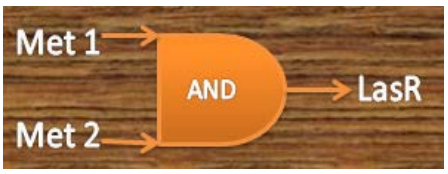
Regulation can be quantified by the "response characteristics", meaning the level of gene expression as a function of the concentration of (activated) TFs. Although we consider protein concentration as a continuous variable, essential features of the response characteristics can often be represented more compactly by a binary "logic function", which specifies whether a gene is "ON" (expressed) or "OFF" (silent, or expressed at basal level) at different extremes of cellular TF concentrations.[1]

That's the objective we pursue. Through coupling the binding sites for different TFs in the promoter region of a gene, we want to be able to control its expression in a logic way. Here we focus on the detailed dynamic description of our genetic logic gates, which are extrinsic to Bacillus subtilis, so that it’s entirely controllable by us.

Heavy metal AND gate

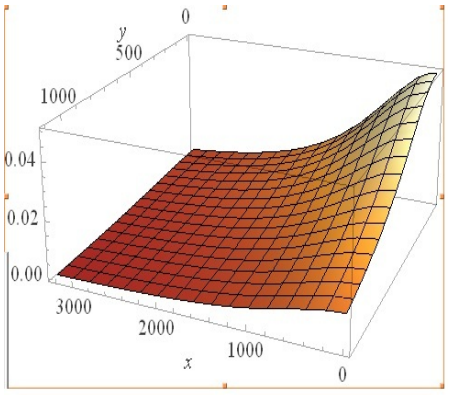
Our system consists in a hybrid promoter which contains binding sites for two transcription factors that act as repressors, CzrA and ArsR. In presence of Met1, CzrA losses affinity for its binding site. Similarly, if there is Met2 in the cell, ArsR also dissociates itself from its binding site. Taking into account these facts, unless Met1 and Met2 are present in the culture, we can say that the genes under this hybrid promoter will be repressed, just like a AND gate should function!

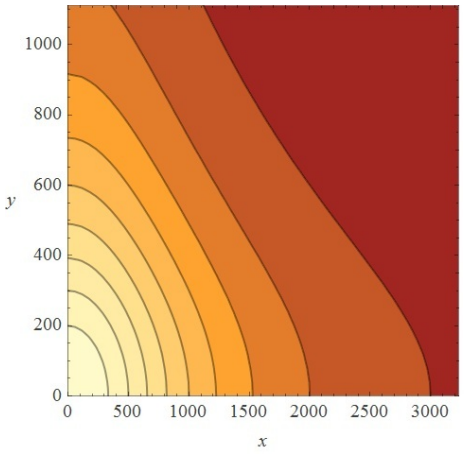
CzrA	ArsR
met1	met2
Zn(II)	As(II)
Cd(II)	As(V)
Co(II)	Cd(II)
Ni(II)	Ag(I)



Repressors

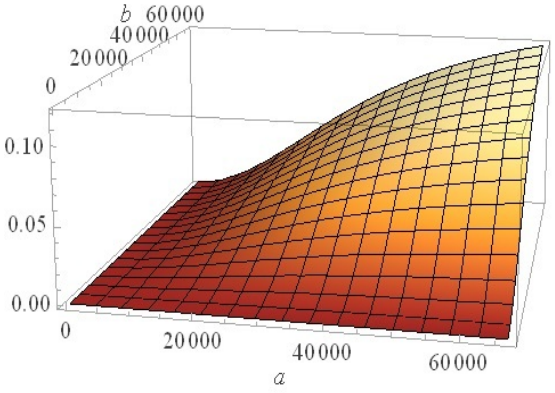
CzrA (x)	ArsR (y)	LasR
0	0	1
0	1	0
1	0	0
1	1	0

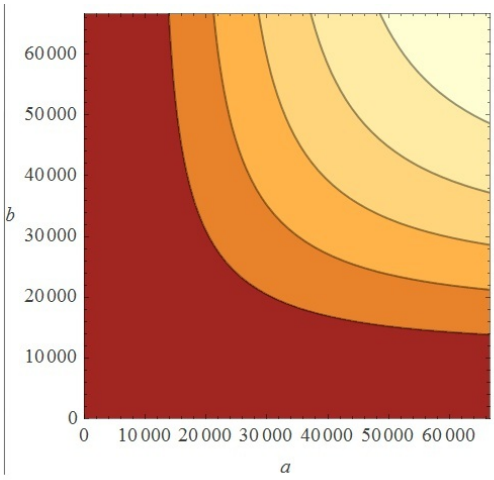




Inputs

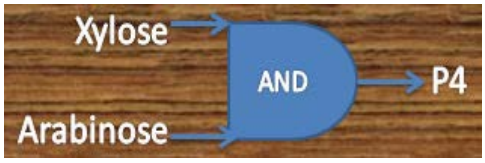
Met 1 (a)	Met 2 (b)	LasR
0	0	0
0	1	0
1	0	0
1	1	1





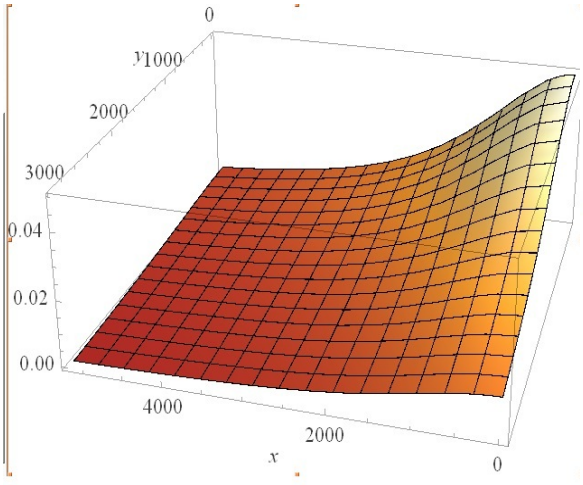
Sweet AND gate

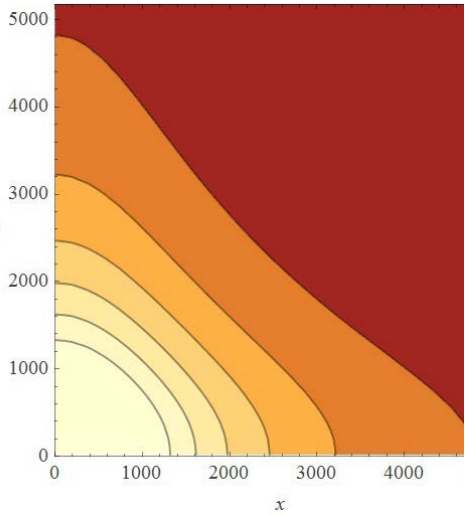
Xylose and arabinose are sugars used by bacteria as carbon sources. XylR and AraC are transcriptional regulators modulated by xylose and arabinose. In this case, the hybrid promoter has as backbone made of the pBAD promoter plus a binding site for XylR. XylR represses the transcription until xylose is present in the cell, the same way that ArsR and CzrA work. AraC represses through the formation of DNA looping. When arabinose enters the cell, it dissolves the loop and transcription starts. We can conclude that arabinose and xylose are essential for the expression of genes under the pBAD/xylR promoter.



Repressors

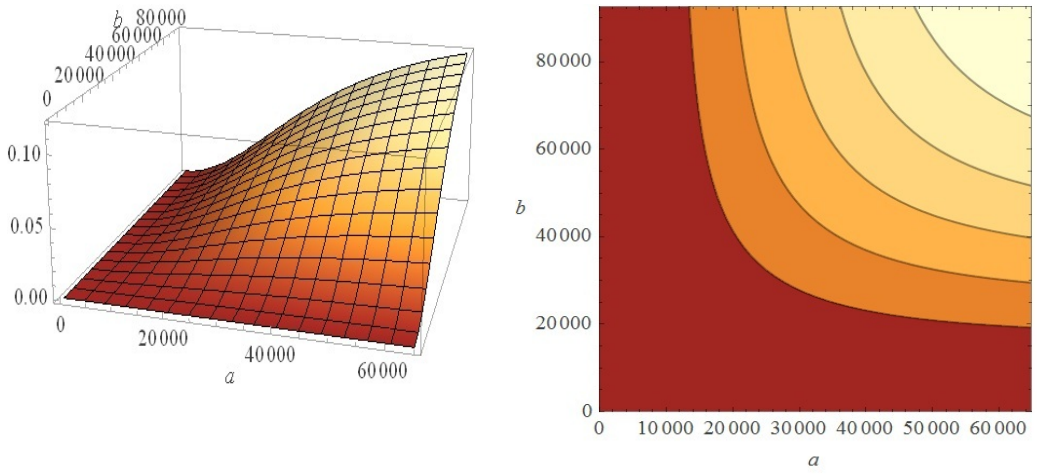
xylR (y)	araC (x)	P4
0	0	1
0	1	0
1	0	0
1	1	0





Inputs

Xylose (b)	Arabinose (a)	P4
0	0	0
0	1	0
1	0	0
1	1	1



The behavior of both ANDs predicted by our model is consistent with what we expected. From the perspective of the repressors, a higher level of PoPs is reached when both repressor concentrations are 0, and PoPs start to fall when the concentration of repressors increases, reaching the highest level of repression (lower PoPs) at high concentration of both repressors. This behavior reminds us of the way a NOR behaves, because at low levels of repressor PoPs is high, but for repression, high concentration of one repressor is enough. However, we can't control (directly) the concentration of repressors, so the right way to look at the system is from the perspective of the Input: the metals or the sugars.

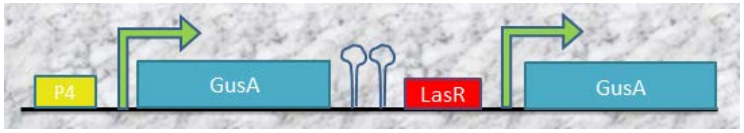
If we looked PoPs as a function of the Input, it behaves as an AND logic gate, as can be seen in the continuous TRUTH TABLE. At lower concentrations of Input, the PoPs level is in a very repressed stage. If you keep adding concentration of one of the Input on only one of the axes (yes, axes is the plural of axis), the activation won't be great until you add high concentrations on the other axis. PoPs will increase as the Input concentration increases. The PoPs increases in a direct proportion fashion.

Ecce OR…hmo

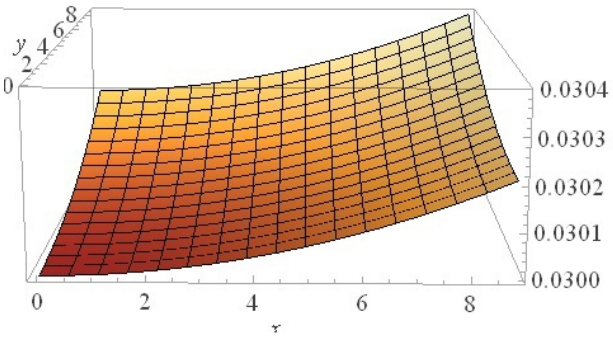
Our Or consists of two promoters (LasR and A3) that have downstream the same gene (GusA and GFP). Each of these promoters is activated by one of the transcription factors encoded in the ANDs. Specifically, these transcription factors are LasR, which activates LasB, and P4, which activates A3.

Now, about the behavior of this construction predicted by our model, it does act as an Or. As seen from the perspective of the transcription factors, activation occurs when any of them is in high

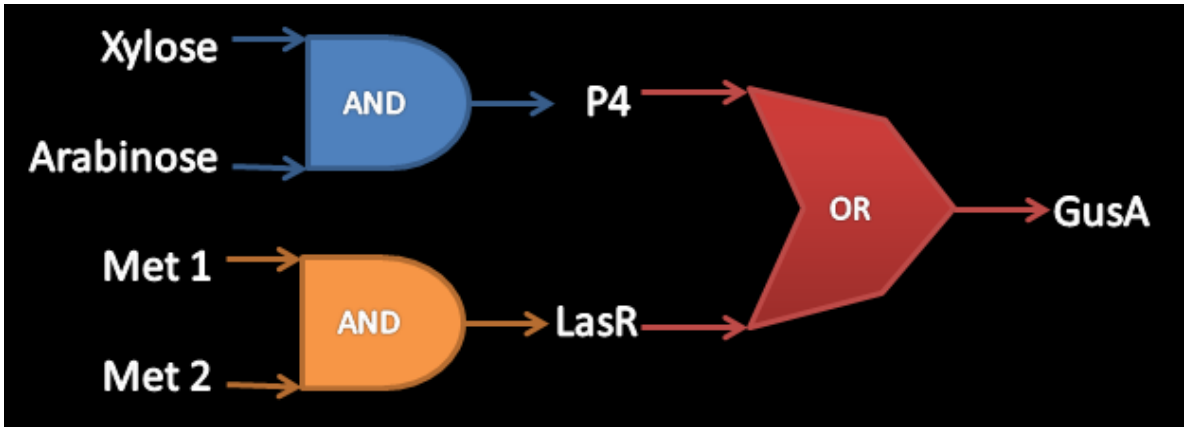
concentrations in the system. As the Or is activated by two different promoters, activation does seem to reach its summit when there are both activators in the system, while the genes are only basally expressed when no activator is present.



Inputs		
P4 (r)	LasR (y)	GusA
0	0	0
0	1	1
1	0	1
1	1	1



System



References:

[1] Buchler et al. On schemes of combinatorial transcription logic
www.pnas.org/cgi/doi/10.1073/pnas.0930314100