Transfer function

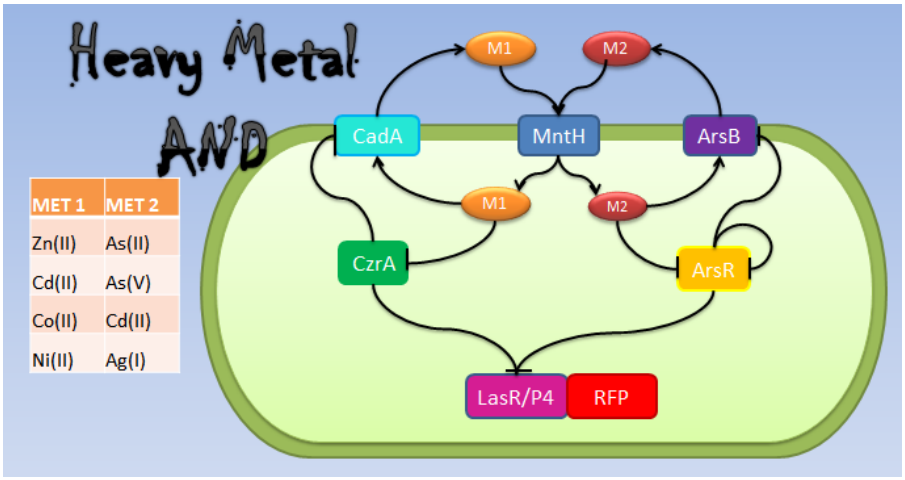
We were interested in modeling the output of our logic gates based on the input. This is called the "Transfer function".

Imagine this process like a black box that will give you the dynamic concentration through time of whatever is downstream of the "AND" when you feed it with input data. It doesn't matter if it's a single input burst or a continuous input.

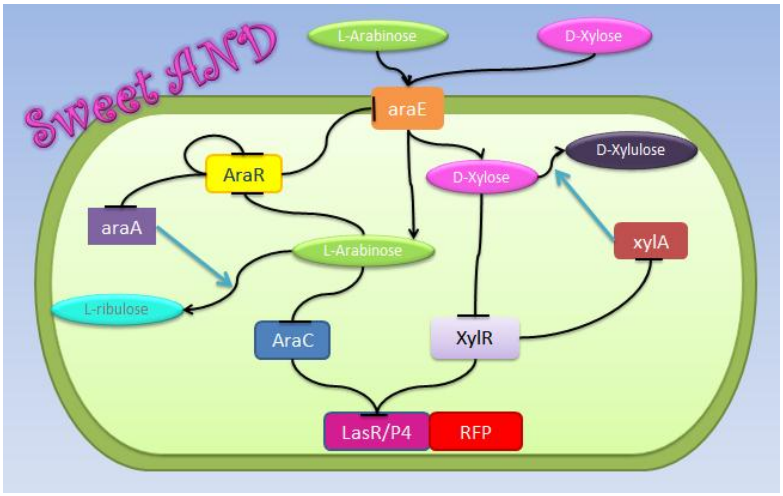
Our logic AND gates are fully dependent on the intracellular concentrations of the inducer (sugars for the SweetAND & metal ions for the HeavyMetalAND).

Then, to accomplish our duty in model, we use the "Transfer Function", for which it is necessary to take into account the regulation and dynamics of the endogenous B. subtilis intake-efflux system that controls the intracellular concentrations of Heavy Metals and Sugars. All this is going to be the "Black Box".

So, our first task was to reconstruct the regulatory network of B. subtilis for both intake-efflux systems. The regulation data was retrieved from several papers and databases like http://bsubcyc.org/



[Heavy metal AND: Some metals are introduced to the cell by MntH. Those metals, in the event of Met 1 being in the cell, are expelled from the cell by a transporter of the CadA operon and those of Met2 set by ArsB. The master regulators of each set are CzrA and ArsR. They control the production of the transporters by repressing them in the absence of metals. Both, CzrA and ArsR repress the expression of LasR or P4 and RFP under the hybrid promoter of CadA+ArsR.]



[Sweet AND gate: Arabinose and xylose are imported into the cell by the araE permease. The carbon metabolism system is highly regulated in order to optimize the consumption of sugars using the least amount of energy. XylR and AraR are repressors responsible for the regulation of genes involved in the metabolism and intake of xylose and arabinose, respectively. Although XylR is not well characterized, some studies suggest a correlation between XylR and the concentration of xylose inside the cell. AraR not only represses the production of genes like araE or araA, but it also represses itself. AraA and xylA are isomerases that convert L-arabinose into L-ribulose and D-xylose into D-xylulose. XylR from B.subtilis is also used by our construction as a repressor, but the production of xylR is the product of the endogenous production plus the production because of a constitutive promoter. Under that constitutive promoter, AraC is also produced for the regulation of the hybrid promoter. The output of the AND is the production of a transcription factor, LasR or P4 and RFP.]

Parameters

We looked for parameters and information of every molecular species in our system to be able to construct a hybrid Genetic-Biochemical network. Then, based on it, we built a model that could predict the behavior and ask questions of the synthetic system coupled to the endogenous system.

Parameters	Description	Value	Source
$\beta_{transc\ i}$	Maximal transcription rate of gene i of length j (nt)	j/80 mol/s	Bremer, H., Dennis, P. P. (1996) Modulation of chemical composition and other parameters of the cell by growth rate. Neidhardt, et al. eds. Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology, 2nd ed. chapter 97a
$\beta_{transl\ i}$	Maximal translation rate of protein i of length j(aa)	j/20 mol/s	Bilgin N, Claesens F, Pahverk H, Ehrenberg M. Kinetic properties of Escherichia coli ribosomes with altered forms of S12. J Mol Biol. 1992 Apr 20 224(4):1011-27
α_{RNA}	Degradation rate of the mRNA of specie i. (Equal for all species)	0.003 mol/s	Yang E, van Nimwegen E, Zavolan M, Rajewsky N, Schroeder M, Magnasco M, Darnell JE Jr. Decay rates of human mRNAs: correlation with functional characteristics and sequence attributes. Genome Res. 2003 Aug13(8):1863-72. doi:10.1101/gr.1272403 p.1864 left column 2nd

			paragraph
α	Degradation rate of proteins (equal for all, from GFP half life)	1.38e-4, 0.0023	- Li X, Zhao X, Fang Y, Jiang X, Duong T, Fan C, Huang CC, Kain SR. Generation of destabilized green fluorescent protein as a transcription reporter. J Biol Chem. 1998 Dec 25 273(52):34970-5. -Megerle, J. 2011.
$V_{max\ CzrA}$	Vmax of production of Czra	70 mol/s	Inference from [9]
$K_{m\ CzrA}$	Km for CzrA production	40 mM	Inference from [9]
$K_{cat\ met\ in}$	Maximum number of imported metal molecules per transporter	600 mol/s	Assumption
$K_{m\ met\ in}$	Km for import of metals	50mM	Assumption
$K_{cat\ met1\ out}$	Maximum number of exported metal molecules per transporter	$= K_{cat\ met\ \text{in}}$	Assumption
$K_{m\ met1\ out}$	Km for export process by any CadA transporter	$= K_{m\ met\ \text{in}}$	Assumption
$K_{cat\ met2\ out}$	Maximum number of exported metal molecules per transporter	$= K_{cat\ met\ \text{in}}$	Assumption
$K_{m\ met2\ out}$	Km for export process by ArsB transporter	$= K_{m\ met\ \text{in}}$	Assumption
$K_{a\ ArsR}$	Afinity constant to its bindinding site	330 nM	Groningen 2009
$K_{a\ arabinose}$	Afinity constant of arabinose to AraR and AraC	AraR= 8.4 µM AraC=50µM	**Procházková, 2012** **Mergele J, 2011**
$K_{a\ xylose}$	Afinity to XylR of xylose	8.4 µM	Assumption (equal to arabinose Ka)
$K_{a\ met1}$	Ka of Me1 for CzrA	6 µM	Groningen 2009
$K_{a\ CzrA}$	Afinity to CadA promoter	330 nM	Assumption (equal to ArsR)
$K_{cat\ AraE}$	Kcat for transport of sugar into the cell.	1000 mol/min	Megerle, J. 2011
$K_{m\ AraE}$	Km for transport of sugar into the cell.	50µM	Assumption from Megerle, J. 2005
$K_{cat\ AraA}$	Kcat for conversion of L-Arabinose into L-ribulose	1943/min	Sabio DB ID:39773 PUBMED: 20688514
$K_{m\ AraA}$	Km for conversion of L-Arabinose into L-ribulose	31.9 mM	Sabio DB ID:39773 PUBMED: 20688514
$K_{cat\ XylA}$	Kcat for conversion of D-xylose into D-xylulose	1943/min	Assumption (equal to AraA)
$K_{m\ XylA}$	Km for conversion of D-xylose into D-xylulose	31.9 mM	Assumption (equal to AraA)

Justification of formalisms

In our project, we use different mathematical formalisms to achieve a superior model. According to our own experience, a simple ODE model is difficult to calibrate if the expected behaviors are not known. Due to the underlying assumption of absolute interaction capabilities, it is easy to ignore important concurrent delays under a simplistic mass-action dynamic. For logic gates, achieving correct time-scales is crucial. Thus, we refine ODE behaviors using a reductionist approach: rule based modeling. Through the use of Kappa, we are able to accurately predict behaviors and thus refine our ODE model. For example, a diffusion limit through membrane channels can be implemented through the use of a Michaelis-Menten dynamic, something that unless we knew was required, would have been very difficult to foresee. Moreover, a reductionist approach evidentiates the mechanistic understanding required to achieve a believable model. For an iGEM project, reductionist understanding of the project is a tremendous advantage when characterizing standardized parts.

However, rule-based models are difficult to scale, not to mention that they are computationally very expensive, and therefore a compact ODE system is still preferable for large-scale simulations and iterations. Consequently, we utilize rule-based models to refine an ODE approximation, with which we can further scan parameters and explore the solution space. In other words, we use different formalisms to take maximum advantage of their respective strengths, avoid their weaknesses, and thus achieve a superior model.

Stochastic and deterministic models

Sometimes people wonder why we used Kappa, a stochastic approach, to model our system, which was also described by deterministic differential equations. First of all, Kappa helped us come up with the concentration ranges entered in the differential equations (here, an exclamation of awe is usually heard). How did this come to happen? Well, the simulation in kappa is driven by the binding events and is dependent of the species concentrations, so using general kinetic rates we are able to see the dynamic behavior given by the architecture of the network. This allowed us to approximate the final species concentration and look for parameters missing in our ODE system, which is something useful, since we were as lost as a three-legged dog in a rodeo dance.

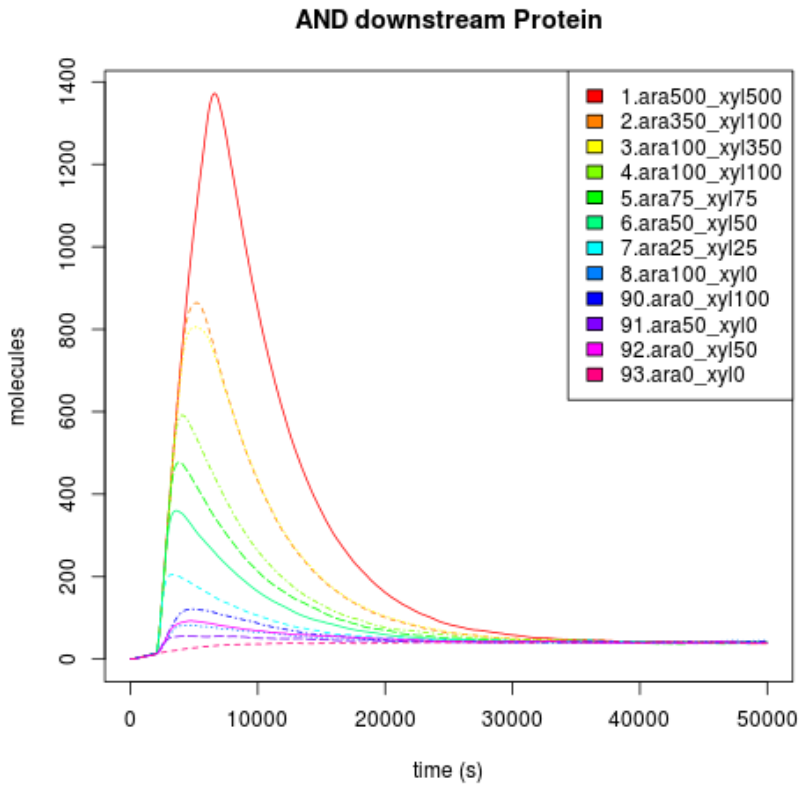
Sweet AND !!

We ran the and_sugar.ka kappa script shifting the sugar initial concentrations in the single input burst and getting the average of 100 iterations to eliminate the noise in the simulation. After running some simulation time, we were happy to see that our "Sweet AND" behaved as expected, as an AND! Besides, we were able to see the maximal concentration of the protein that would be downstream of our AND, and the time it took to reach it. As you can see, our simulation grid is not described in its entirety. This is because of the high kappa simulation time consumption, so we chose to feed our ODE system with some parameters and scan the system in a broader manner

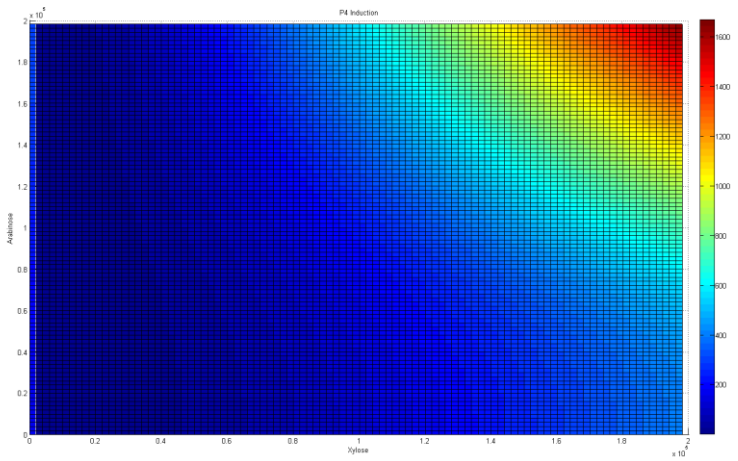
instead of dying of old age waiting for Kappa. Nevertheless, some behaviors can be noticed only looking at the simulation grid, for example, the activation of P4, depending on the xylose and arabinose concentrations. As it becomes apparent through a meticulous and insightful analysis only doable by a really smart, handsome, and admirable person, the activation of P4 depends on the concentrations of both, xylose and arabinose. When only one of the two sugars has a high concentration, the activation is not as elevated as when the two have high concentrations.

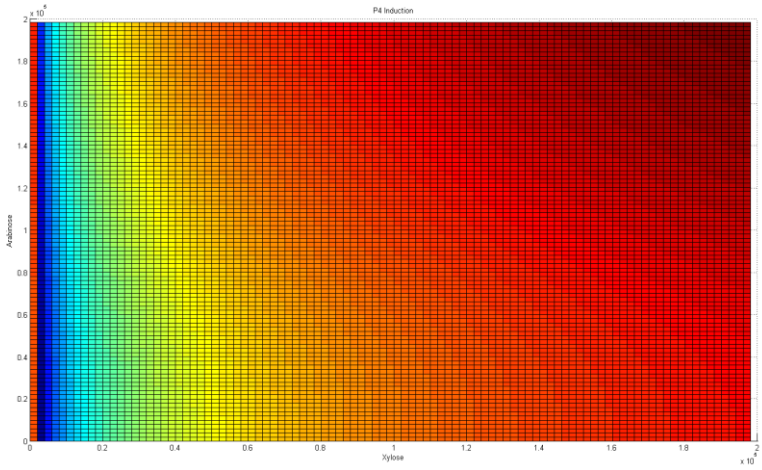
//grid de hector//

This plot describes the time to reach steady state and maximal concentration of the AND downstream protein. It also describes what the maximal concentration of the AND protein is. All of it is based on a single input burst concentration.



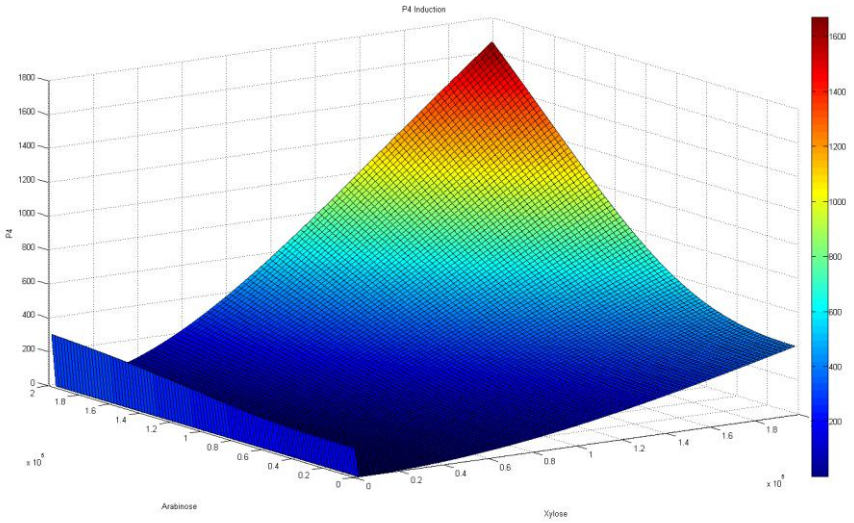
Then, with the ODE system ready to rock in matlab, we scanned for different combinations of sugar concentrations. Even though we looked at a slightly different contribution of the inputs (xylose has a stronger activation effect over the Sweet AND system), meaning that our system had a different behavior when looking at the endogenous system in B. subtilis than just looking at our synthetic construction, our system still worked as an AND. This is reflected by the bi-dimentional data analysis. Nevertheless, this is not true for the heavy metal AND.





There came a time where we put together our wits to analyze the three dimensional plot. It was at this time that we found out that some of us were not as brilliant as we thought, but that is a tale for another moment, right now we'll just tell the tale of our observations and conclusions.

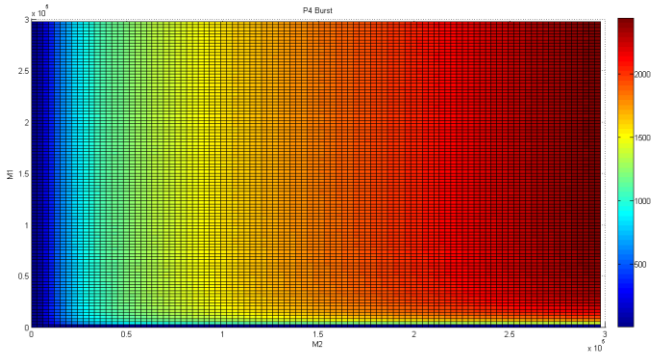
Our first observation worthy of mention came when we realized that the system was most repressed when there was scarce xylose in the system, not when there was none. On the contrary, when there is no xylose in the system, there is a very small expression gradient dependent on the concentration of arabinose, yet this gradient does not surpass a threshold where we could consider the AND as "ON".

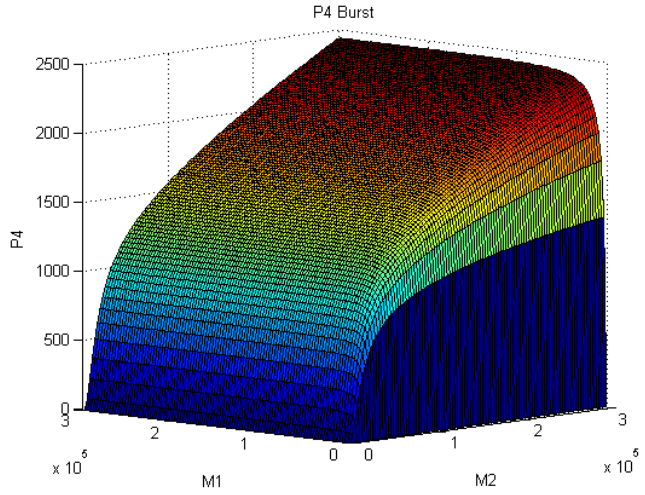


We are coupling an endogenous and an exogenous repressor to our AND. This approach can easily be explained by the fact that we are expressing the endogenous repressor XylR in a constitutive manner. This is due to the fact that it has been reported that it has to be in high concentrations in order to repress in an effective manner. When we add xylose to the medium, endogenous XylR gene increases its expression, (as a super saiyan increases its ki). This makes repression against the AND increase when xylose is found in the medium in small amounts. After the repression threshold is surpassed, xylose stops being a repressor and shifts to being an activator, so that an increase in its concentration augments the AND's downstream protein expression.

Metal AND

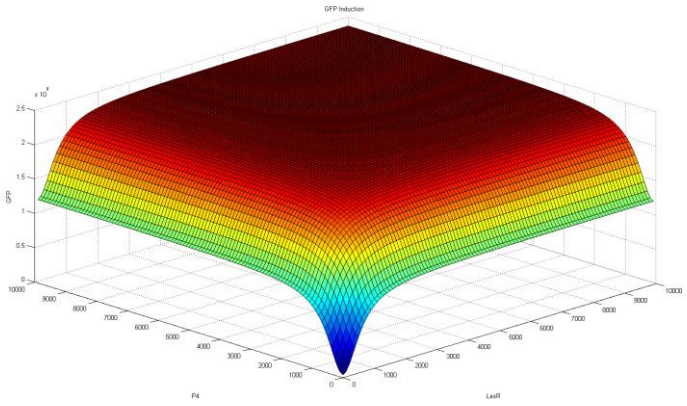
Metal homeostasis in the cell involves an effective response in order to withstand possible stress conditions in which high concentrations of heavy metals are involved. Both approaches, ODEs with Matlab and the stochastic one with kappa, give us similar results. First, we were able to see that the AND response is not as sensitive to higher concentrations. For the AND to have a good performance, low concentrations of Met 1 and 2 are required. As we said, we infer that metal response is a fast process. The accumulation of the output protein shows a sudden increase in production resulting from a very small change in metal concentrations. Another remarkable fact is about the role each TF (CzrA and ArsR) has. Since ArsR represses itself, its production has a succession of spikes. Nevertheless, CzrA increases its production if the metal concentration within the cell also increases, but it doesn´t have a drop in production because their decay depends only on the dilution and degradation of the protein. That way, the effects of CzrA are more notable than ArsR. This is reflected in a greater contribution of Met1 in the regulation of the AND promoter than that of Met2.





OR

Assuming that both transcription factors had the same affinity, both the stochastic and the deterministic approach show us that its behavior is not dislike an Or gate.

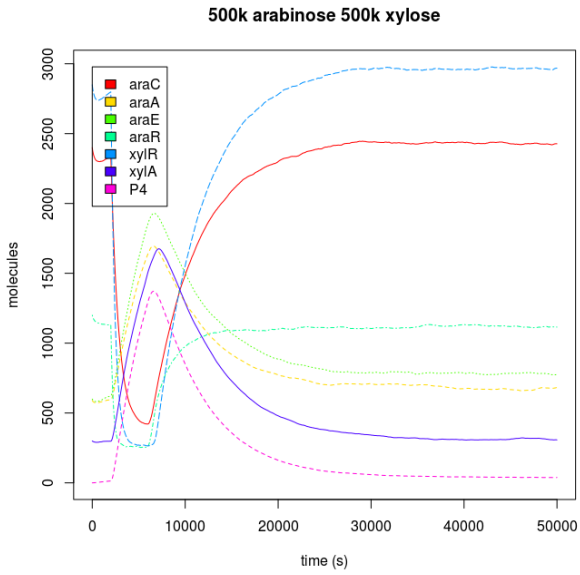


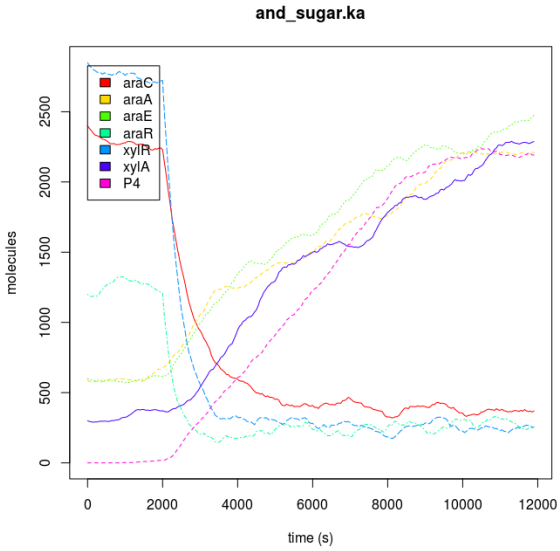
Effects of the metabolite's nature

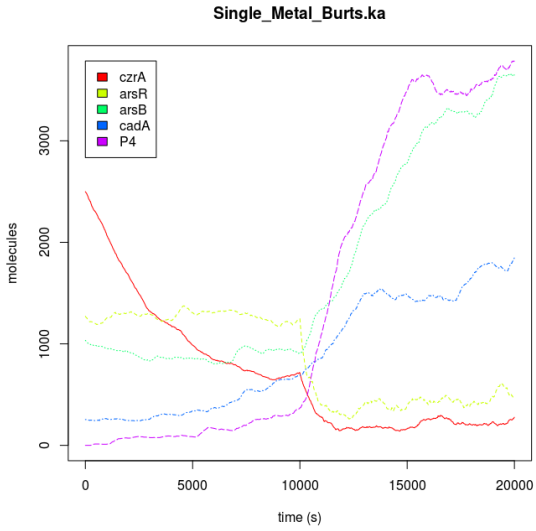
The inputs used by each AND have a unique effect in the response of the cell, because carbohydrates are degraded by the cell. In contrast, metals just reach an equilibrium and not-toxic concentration within the cell through exporting it outside the cell, maintaining the concentration constant.

When we add sugars to the sweet AND system, if it is added just like a discrete and unique dose of it, the cell with react to the alteration through the activation of degradation machinery, but when the sugar pool is empty, the cell will achieve a similar state to the one before the sugar input. When the sugar addition is constant, the cell has a complete phenotype change, reaching a new steady state.

When we look at the behavior of the metals AND, we find that it is similar to the one reached by a constant dose of arabinose, because the metals levels in just one dose will be constant since metals can´t be degraded.







Conclusions

After making the model just for our AND, me coupled the endogenous Bacillus subtilis system to our predictions. After this, we found out that the predictions given by both assumptions were not the same, proving that the endogenous system was, in fact, an important matter to consider, so we considered it. Obviously, as the model that did not take into account the endogenous system was farther away from the actual system, we opted for using the model that did take it into account, making our transfer function a more realistic representation of the system, in spite of the fact that we could not find all the parameters.

Some of these parameters, unfortunately, were the diffusion within the nanotubes, and the amount, size, form, frequency of creation, or any other useful data of nanotubes you could think of (luckily for us, we could not think of that many). Even though we had to face all these problems,

with the little light shone by people before us onto the spectral essence of the nanotubes, it was possible for us to say that our system can, in theory, simulate Boolean operations.

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