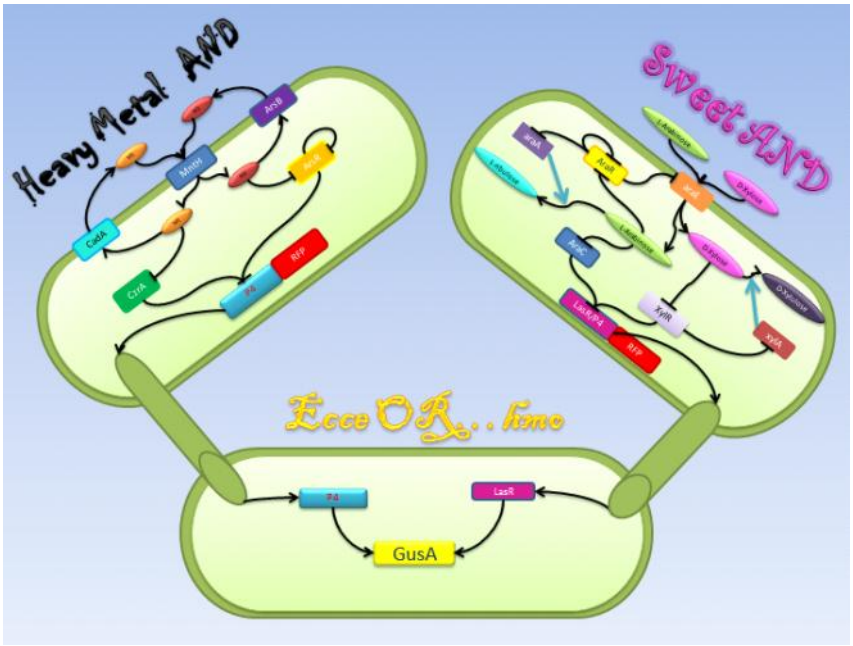
ODEs model description and assumptions



Our team made both, a deterministic and a stochastic approach. Here, we are going to describe the deterministic model.
This model's scope is single cell. While the regulation steps were modeled through Hill equations, other reactions were treated under Michaelis-Menten assumptions, notably isomerizations and transport processes (i.e. those that involved energy consumption).
General assumptions:

- The maximal transcription rate for the system species was obtained by dividing the maximum number of nucleotides processed by the RNA polymerase (i.e. maximum polymerase activity), by the length of the nucleotide sequence.
- Translational rates were obtained with the maximum number of amino acids added by the ribosome divided by the length of the protein in amino acids.
- The protein degradation rate is considered as equal for all the protein species in the model.
- The degradation rate for mRNAs is equal for all the different species of mRNAs.
- The different binding sites of the same transcription factor have the same affinity to the TF.
- For each mRNA species, its concentration will be the sum of the production as affected by its respective promoter and the transcription factors that regulates it, minus the degradation of the mRNA in the time.
- For each protein, the change in its concentration depends in the amount of protein produced by translation minus the degradation rate of the protein.
- The Hill coefficients tend to be 2.
- The parameters of concentration were expresed in terms of molecules per cell(Vol of a B. subtilis cell ~10^-14) and the time units were converted sec.

Heavy metal AND

In Bacillus subtilis, many metal ions, both essential, and others like arsenic, are introduced in the cell by the ion pump MntH. We make two assumptions, first, that the used metals were imported by MntH and second, that all the metals introduced by MntH (especially those that we use for our AND gate) have the same intake rate. Met1 is pumped out of the cell by a transporter of the CadA operon (most of them require ATP hydrolysis), and Met2 by ArsB (Moore C, 2005[21]). In that way, each metal's concentration in the cell was defined by Michaelis-Menten equations as the amount of introduced metal by each MntH molecule in the cell, minus the amount of exported metal by its respective transporter, assuming that all the metals imported or exported by the same transporter had the same affinity to this transporter and had the same chance to pass into the cell.

Metal flux

$$\frac{\mathrm{d}met_1}{\mathrm{d}t} = \frac{K_{cat\,met1\,in}\,MntH\;met_{1\,out}}{k_{m\,met1\,in}+met_{1\,out}} - \frac{k_{cat\,met1\,out}\,CadA\;met_1}{k_{m\,met1\,out}+met_1}$$

$$\frac{\mathrm{d}met_2}{\mathrm{d}t} = \frac{K_{cat\,met2\,in}\,MntH\;met_{2\,out}}{k_{m\,met2\,in}+met_{2\,out}} - \frac{k_{cat\,met2\,out}\,ArsB\;met_2}{k_{m\,met2\,out}+met_2}$$

The transcription factors which are involved in the AND processing are CzrA and ArsR. ArsR is repressed by itself when there are no Met2 ions. We use a Hill function to express the mRNA production. The function shows the effect of the binding of ArsR in its promoter. This way, the repression effect will be affected by the afinity of ArsR to the binding site and the concentration of ArsR. About CzrA, there are studies that suggest that the total amount of the protein in the cell is correlated with the levels of the metals that it tends to expell (Moore C, 2005 [9]). To describe this phenomenon, we defined the production of the mRNA of CzrA as the production due to intracellular Met1 mediated induction into the cell through a Michaelis-Menten relation.

ArsR and CzrA production

$$\frac{\mathrm{d}Rna_{ArsR}}{\mathrm{d}t} = \beta_{transc\,ArsR}\left(\frac{1}{1+(\frac{ArsR^*}{K_{a\,ArsR}})^n}\right) - \alpha_{Rna\,ArsR}\;Rna_{ArsR}$$

$$\frac{\mathrm{d}ArsR}{\mathrm{d}t} = \beta_{transl\,ArsR}\;Rna_{ArsR} - \alpha_{ArsR}\;ArsR$$

$$ArsR^* = \frac{ArsR}{1+(\frac{arabinose}{K_{a\,arabinose}})^n}$$

$$\frac{\mathrm{d}Rna_{CzrA}}{\mathrm{d}t} = \frac{V_{max\,CzrA}\,Met_1}{K_{m\,CzrA}+Met_1} - \alpha_{RnaCzrA}\;Rna_{CzrA}$$

$$\frac{\mathrm{d}CzrA}{\mathrm{d}t} = \beta_{transl\,CzrA}\,(Rna_{CzrA}) - \alpha_{CzrA}\;CzrA$$

$$\mathrm{l}\;CzrA^* = \frac{CzrA}{1+(\frac{met_1}{K_{a\,met1}})^n}$$

Finally, the proportion of active transcription factors (unbound to their respective metals) are defined with a hill function as the fraction of the total protein plus the relationship between the affinity constant of the binding and the amount of metal. The higher amount of metal or the afinity of it to the binding with TF corresponds with the lower amounts of active TF. The transporters for Met1 and Met2 are also under the regulation of ArsR and CzrA; the CadA operon is repressed by CzrA, and ArsB is repressed by ArsR.

CadA y ArsB transporter

$$\frac{\mathrm{d}Rna_{CadA}}{\mathrm{d}t} = \beta_{transc\,CadA}\left(\frac{1}{1+(\frac{CzrA^*}{K_{CzrA}})^n}\right) - \alpha_{Rna\,CadA}\;Rna_{CadA}$$

$$\frac{\mathrm{d}\,CadA}{\mathrm{d}t} = \beta_{transl\,CadA}\;Rna_{CadA} - \alpha_{CadA}\;CadA$$

$$\frac{\mathrm{d}Rna_{ArsB}}{\mathrm{d}t} = \beta_{transc\,ArsB}\left(\frac{1}{1+(\frac{ArsR*}{K_{a\,ArsR}})^n}\right) - \alpha_{Rna\,ArsB}\;Rna_{ArsB}$$

$$\frac{\mathrm{d}ArsB}{\mathrm{d}t} = \beta_{transl\,ArsB}\;Rna_{ArsB} - \alpha_{ArsB}\;ArsB$$

The output of the AND operation is the production of one of two reporters, P4 or LasR respectively. The production of the reporter mRNA depends on the status of its promoter, which has a state space of being bound to ArsR, to CzrA, to both, or bound to none. This approach was made supposing that

the binding-mediated repression of each TF is independent, and therefore the cumulative intensity of repression by ArsR and CzrA is the product of their independent repressions.

AND output P4/LasR

$$\frac{\mathrm{d}Rna_{P4}}{\mathrm{d}t} = \beta_{transc\,P4}\left(\frac{1}{1+(\frac{CzrA^*}{K_{a\,Czra}})^n}\right)\left(\frac{1}{1+(\frac{ArsR^*}{K_{a\,ArsR}})^n}\right) - \alpha_{Rna\,P4}\ Rna_{P4}$$

$$\frac{\mathrm{d}P4}{\mathrm{d}t} = \beta_{\,transl\,P4}\ Rna_{P4} - \alpha_{P4}\ P4$$

$$\frac{\mathrm{d}Rna_{LasR}}{\mathrm{d}t} = \beta_{transc\,LasR}\left(\frac{1}{1+(\frac{CzrA^*}{K_{a\,Czra}})^n}\right)\left(\frac{1}{1+(\frac{ArsR^*}{K_{a\,ArsR}})^n}\right) - \alpha_{Rna\,LasR}\ \ Rna_{LasR}$$

$$\frac{\mathrm{d}LasR}{\mathrm{d}t} = \beta_{transl\,LasR}\ Rna_{LasR} - \alpha_{LasR}\ LasR$$

Sweet AND

Xylose and arabinose are part of the pool of molecules that can be used by B. subtilis as carbon sources. Amongst membrane transporters, affinity for particular kinds of sugars is often high; nevertheless AraE is the main permease responsible for the intake of arabinose, xylose, and galactose monosaccharides. AraE is down-regulated by AraR, which also represses some other genes involved in arabinose metabolism. AraR is inactivated by arabinose and by itself (Krispin O, 1998 [19]). These little details evidentiate the fact that arabinose is required for xylose cellular influx. The concentrations of XylA and AraA depend on the concentration of their respective repressors, XylR and AraR, and their respective dissociation constants.

Transport and metabolism proteins

$$\frac{\mathrm{d}Rna_{AraA}}{\mathrm{d}t} = \beta_{transc\,AraA}\ \frac{1}{(1+(\frac{AraR*}{K_{a\,AraR}})^n)}\ - \alpha_{Rna\,AraA}\ Rna\_AraA$$

$$\frac{\mathrm{d}Rna_{AraE}}{\mathrm{d}t} = \beta_{transc\,AraE}\left(\frac{1}{1+(\frac{AraR^*}{K_{a\,AraR}})^n}\right)\ - \alpha_{Rna\,AraE}\,Rna_{AraE}$$

$$\frac{\mathrm{d}Rna_{XylA}}{\mathrm{d}t} = \beta_{transc\,XylA}\ \frac{1}{(1+(\frac{XylR*}{K_{a\,XylR}})^n)}\ - \alpha_{Rna\,XylA}\ Rna_{XylA}$$

$$\frac{\mathrm{d}XylA}{\mathrm{d}t} = \beta_{transl\,XylA}\ Rna_{XylA}\ - \alpha_{XylA}\ XylA$$

$$\frac{\mathrm{d}Rna_{AraC}}{\mathrm{d}t} = \beta_{transc\,AraC}\ - \alpha_{Rna\,AraC}\ Rna_{AraC}$$

$$\frac{\mathrm{d}AraC}{\mathrm{d}t} = \beta_{transl\,AraC}\ Rna_{AraC}\ - \alpha_{AraC}\ AraC$$

Intracellular xylose induces the production of genes involved in its metabolism, also through the inactivation of their correspondent repressor, XylR. XylA is the enzyme that catalyzes the conversion of D-xylose to D-xylulose. For arabinose, the isomerase that catalyzes the conversion of L-arabinose into L-ribulose is AraA(Gu Y, 2010 [20]).
In that way, the amount of intracellular arabinose is defined as the amount introduced by AraE minus the amount converted to L-ribulose by AraA; for xylose the process is similar but the degradation depends on xylA. We assume that the rates of intake of both sugars by AraE are similar. For the degradation rates by AraA and XylA, they are defined by Michaelis-Menten kinetics, since this kind of equation takes into account the energy consumption, and also because it is a simple reaction with one step and one substrate.

Sugar uptake

$$\frac{\mathrm{d}\,arabinose}{\mathrm{d}t} = \frac{\beta_{upt\,arabinose}\,AraE\;arabinose}{K_{m\,AraE}+AraE} - \frac{K_{cat\,AraA}\,AraA\;\;arabinose}{arabinose+K_{m\,AraA}}$$

$$\frac{\mathrm{d}\,xylose}{\mathrm{d}t} = \frac{\beta_{upt\,arabinose}\,AraE\;arabinose\,/}{K_{m\,AraE}+AraE} - \frac{K_{cat\,XylA}\;\;XylA\;\;xylose}{(\,xylose+K_{m\,XylA}}$$

As for the amount of XylR presented in the system, we had two sources of production. One is the endogenous production in Bacillus subtilis, while the other is the production by the exogenous construct introduced in the genome under the Pveg constitutive promoter. This construction was introduced to counteract the leakiness of the promoter under XylR repression [BBa_K143036]. For AraR, which is produced only through the endogenous B. subtilis production, we considered the self-repression and described its production through a Hill function dependent on the concentration of the protein (Sá-Nogueira, 1997).

AraR and XylR TF production

$$\frac{\mathrm{d}\,Rna_{AraR}}{\mathrm{d}t} = \beta_{transcAraR}\,(\frac{1}{1+(\frac{AraR^*}{K_{AraR}})^n}) - \alpha_{Rna\,AraR}\;Rna_{AraR}$$

$$\frac{\mathrm{d}AraR}{\mathrm{d}t} = \beta_{transl\,AraR}\;Rna_{AraR} - \alpha_{AraR}\;AraR$$

$$AraR^* = \frac{AraR}{1+(\frac{arabinose}{K_{arabinose}})^n}$$

$$\frac{\mathrm{d}Rna_{XylR}}{\mathrm{d}t} = \beta_{transc\,XylR}\,(1+q_{xylR}\,xylose\,) + beta_{transc\,XylR\,Pveg} - \alpha_{Rna\,XylR}\;Rna_{XylR}$$

$$\frac{\mathrm{d}XylR}{\mathrm{d}t} = \beta_{transl\,XylR}(Rna_{XylR}) - \alpha_{XylR}\;XylR$$

$$XylR^* = \frac{XylR}{1+(\frac{xylose}{K_{xylose}})^n}$$

The final TF involved in our system is AraC, from E. coli, which is produced in our construct by the Pveg promoter. Its concentration depends exclusively on the maximal rate of transcription minus the general degradation rate. Since the repression method used by AraC is DNA looping, we used the same approach as in (Megerle J, 2011), where the repression intensity of AraC was defined in terms of arabinose instead of AraC concentration. In this case, the Hill coefficient used was n=3, as reported in the same source as the best fitting value. Under the same assumption of independence between the two TF involved in the hybrid promoter, the total repression intensity is the repression given by AraC DNA looping plus the intensity of XylR, both modeled with a Hill function.

LasR/P4 expression

$$\frac{\mathrm{d}Rna_{LasR}}{\mathrm{d}t} = \beta_{transc\,LasR}\,\frac{arabinose^n}{K^n_{a\,arabinose}+arabinose^n}\,(\frac{1}{1+(\frac{XylR^*}{K_{a\,XylR}})^n}) - \alpha_{Rna\,LasR}\;Rna_{LasR}$$

$$\frac{\mathrm{d}LasR}{\mathrm{d}t} = \beta_{transl\,LasR}\ Rna_{LasR}\ -\ \alpha_{LasR}\ LasR$$

$$\frac{\mathrm{d}Rna_{P4}}{\mathrm{d}t} = \beta_{transc\,P4}\,(\frac{1}{1+(\frac{AraC^*}{K_{a\ AraC}})^n})\,(\frac{1}{1+(\frac{XylR^*}{K_{XylR}})^n}) - \alpha_{Rna\,P4}\ Rna_{P4}$$

$$\frac{\mathrm{d}P4}{\mathrm{d}t} = \beta_{transl\,P4}\ Rna_{P4}\ -\ \alpha_{P4}\ P4$$

Ecce OR..hmo

Ok, this is the good one. For the final step, we had two pretty AND gates that had a special response for certain states of their inputs, but we wanted more. So, how could we make a bigger circuit? The trivial answer, obviously, was to wire everything together under a NEW gate! The new one makes an OR operation, where its inputs are the previous gates' outputs. Both outputs, P4 and LasR are reported activators for the promoters A3 and LasB. In that way, our OR consists of two independent promoters that have the same reporter protein downstream: GusA.
We decided to use the Hill function designed for activators. Being additive events, the production of the reporter mRNA will be the sum of the productions by each promoter, as defined by their respective affinities and intracellular concentrations (Bintu L, 2005 [15]).

$$\frac{\mathrm{d}Rna_{GusA}}{\mathrm{d}t} = \beta_{GusA\,P4}(\frac{(1+(\frac{P4}{K_{P4}})^n\omega)}{1+(\frac{P4}{K_{P4}})^n}) + \beta_{GusA\,LasR}\ (\frac{(1+(\frac{LasR}{K_{LasR}})^n\varphi)}{1+(\frac{LasR}{K_{LasR}})^n}) - \alpha_{Rna\,GusA}\ Rna_{GusA}$$

$$\frac{\mathrm{d}GusA}{\mathrm{d}t} = \beta_{transl\,GusA}\ Rna_{GusA}\ -\ \alpha_{GusA}\ GusA$$