MODELING

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OBJETIVE

The final aim was to construct and simulate our system in Symbiology-MatLab and to generate for each module a matrix which considers time, photon/s and PoP/s.

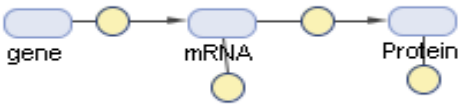
METODOLOGY

1. Construction and simulation of the constitutive elements to simplify each module

It was considered as constitutive species the ones that are not affected by an input and have reached a steady state level before each module is turn on.

Receptors		
Blue	Green	Red
LovTap	CcaS	Cph8_EnvZ
CI	CcaR	OmpR
		CI
Emitters		
Blue	Green	Red
LuxD	LuxD	LRE
LuxC	LuxC	
LuxE	LuxE	
Lumazine	LuxY	

For each specie a simple set of reactions were constructed, these considered the transition for a gene, regulated with a promoter of define strength and which amount along the all simulation keeps constant, to mRNA and finally to a protein; common degradation rates were also considered for the mRNA specie and the protein specie. The kinetic law implemented was Mass-Action it fits well to our system and with the idea to simplify the model.



1.1 General data

a) Promoter strength

The promoter strength for each specie was considered according to the molecular behavior expected for the modules, in silico and in vivo. It was used the promoter family J23 from the iGEM2006_Berkeley team and as the consensus promoter the J23101 based on the literature[1]. For promoter with information not available the media strength for the J23 family was used.

b) Parameters

The standards rates for transcription, translation and degradation (mRNA and protein) were used as parameters for the reactions.

transcription	80bp/sec
Translation	40aa/sec
dmRNA	0.0023105bp/sec
dProtein	1.9254E-5aa/sec

c) Simulations

[1] Measuring the activity of BioBrick promoters using an in vivo reference standard, Jason R Kelly et al.

The simulations were done twice with two different kind of solvers: ode45, a deterministic solver, and stochastic. The time for each simulation was set until the steady state of the species, approximately at 500000 seconds. It would be used the information obtained from the stochastic simulations because it represents a solution for the *Master Chemical Equation.*

1.2 Modules

1.2.1 Receptors

Blue

The blue receptor has two constitutive modules: LovTap and CI. LovTap was simulated with four different promoters: J23117, J23114, J23105 and J23102; which represents weak and medium promoters. The promoter that regulates CI is pTrpL which strength is not reported.

Green

CcaS and CcaR, the constitutive species for the green receptor, were simulated with a weak promoter: J23117.

Read

For the Cph8_EnvZ specie any promoter had been chosen, it was decided to try with promoters of different strength: J23100 (strong), J23107 (medium) and J23112 (weak). The OmpR specie is regulated by OmpC which strength information was not available, the mean strength was used.

1.2.2 Emitters

Blue

For species were considered has constitutive. LuxCDE were simulated with a medium promoter strength according to the information for the pBAD wild-type promoter [Part:BBa_I13453]. The Lumazine specie was simulated with two different medium promoters: J23101 and J23102.

Green

The data obtained from the Blue emitter for LuxCDE was used. LuxY was simulated with two medium promoters: J23101 and J23102.

Red

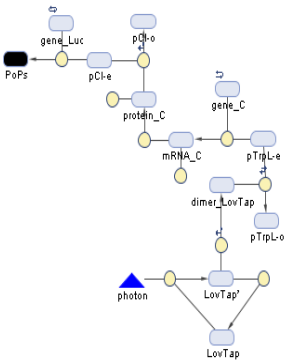
LRE was simulated with a weak promoter: J23100.

2. Construction and simulation of each module

A general approach was followed, make the modules the most simple as possible, it that way all the reactions follow a kinetic law of Mass-Action. For each module a different set of species, amounts and parameters were used, most of them obtained from literature other inferred. The simulations were done with the default solver, sundials, because the ode45 or stochastic solvers were impossible to run. Scan was also used to give the inputs in 100 steps, for each input a simulation was run of 100000 seconds.

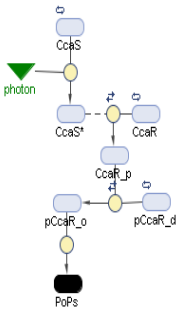
Blue Receptor

It was constructed with 14 species, 10 reactions and 13 parameters. Initial amounts were given for: LovTap, pCIo, pTrpLo and protein_CI.



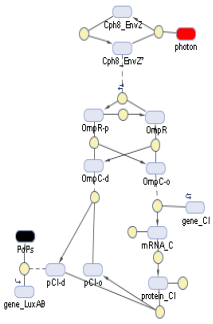
Green Receptor

The green receptor was constructed with 9 species, 4 reactions ans 6 parameters. Initial amounts were given to CcaS, CcaR and pCcaR-e.



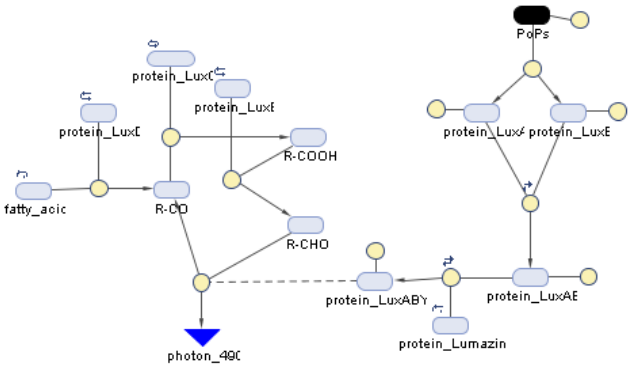
Red Receptor

15 species, 13 reactions and 14 parameters form the red receptor. Initial amounts were given to Cph8_EnvZ, OmpC-o, OmpR-p, pCI_d and protein_CI.



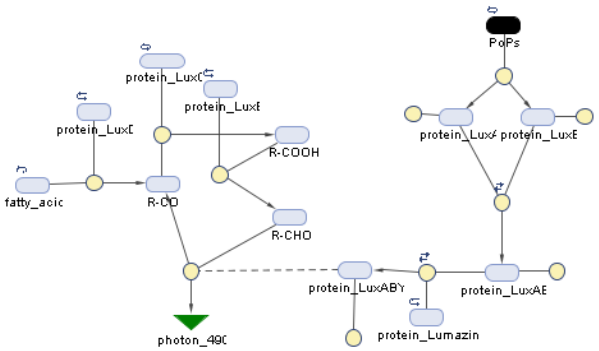
Blue Emitter

The blue emitter is constructed with 15 species, 12 reactions and 14 parameters. Constant amount were given for protein_LuxD, protein_LuxE, protein_LuxC, protein_Lumazine and fatty-acid.



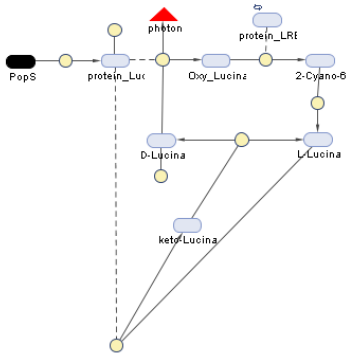
Green Emitter

The green emitter is very similar to the blue one, it is constructed with 14 species, 11 reactions and 13 parameters. Constant amount were given for protein_LuxD, protein_LuxE, protein_LuxC, protein_LuxY and fatty-acid.



Red Emitter

10 species, 8 reactions and 8 parameters form the red emitter. Constant amount was given for protein_LRE and initial one to Luciferin.



3. Lag simulations for receptors

To get a better picture of our module, it was decided to run the simulations with a delay of 25% of the total simulation time which represents the previous dark-sate for the receptors.

4. Matrix construction

The cells arrays got from the simulations were filtered to obtain a matrix with the following information:

a) Receptor: *time, photon, pops.*
b) Emitter: *time, pop, photons.*

The .mat code is available in the iGem-Dropbox. This code get the time, photon and pop data and collapse them in a matrix. For the *pops*|*photons* data get the derivative, the way it chance in the time.

RESULTS

Constitutive elements

The simulations for the constitutive species give the steady state data that would be use as constant concentrations in the simulations for each module. Some of the species were simulated with more than one promoter strength, where the steady state data more useful for each module, according to the biological constraints, would be choose. It is important to say that the data obtained was almost equal in both solver options.

Blue receptor

Modules

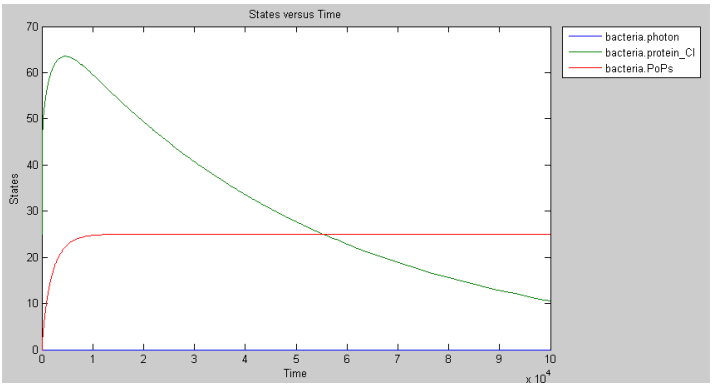
For each simulation a cell array was obtained which includes the changes in the time from an input to the system. The inputs never were constant, just and initial amount was given; two different types were used: photons for the receptors and PoPs for the emitters. The photon input for all the receptors was set as the amount of photons a LED can produce, assuming that all the photons can reach one liter culture within a distance of 3cm and a ratio of 4 cm for a bacteria in a stationary face. The PoPs input data for the emitters was obtained from each receptor simulation but after some simulations the input was reduced to a range of 0:100 PoPs because the module emitters were too sensitive and prone to saturation with higher amounts of input.

In order to obtain the derivative none of the systems have degradation reactions for their outputs, that's why the modules keep in a increasing rate, however if degradation reactions are included, at least for the receptors, the system can reach a basal state.

Lag receptors

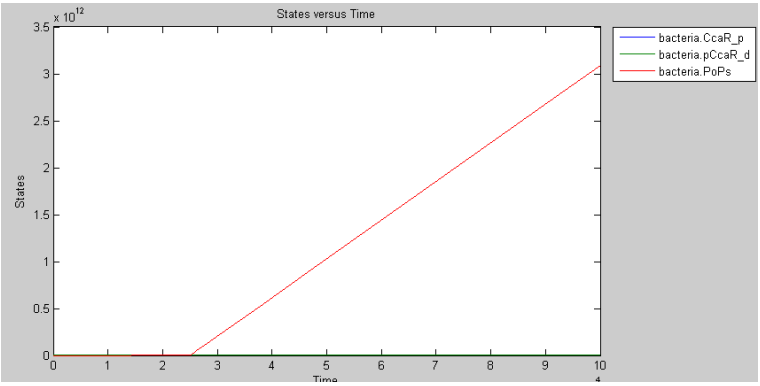
When the simulations were run with a delay period, it was possible to notice some interesting.

a) Blue receptor



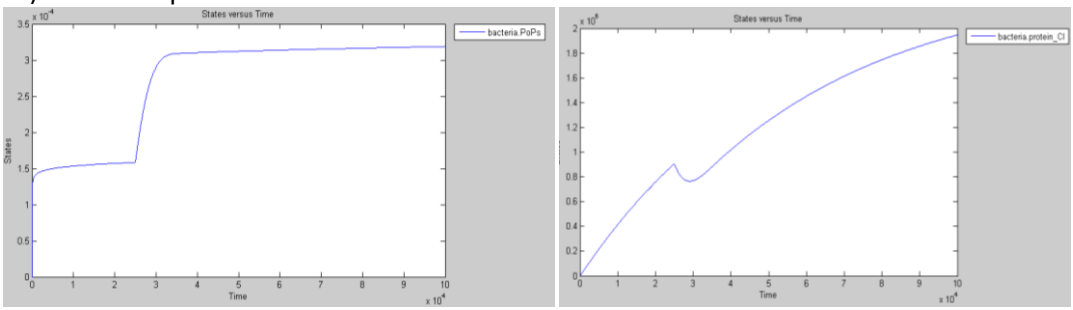
The amount of protein_CI decreased too slowly but it does not need to be 0 to obtain an output: PoPs. The amount of PoPs increase once the system gets the input and reaches a steady state almost immediately.

b) Green receptor



It has no constraints, its behavior is linear.

c) Red receptor



The amount of PoPs obtained from the red receptor was the lowest for the three receptors because of the protein_CI increasing. The size of the input can destabilize the amount of protein_CI but just for a short period of time; despite this the amount of PoPs reach a steady state. Also a basal production of PoPs is noticed.

Matrixes

The matrixes can be found in the iGem-Dropbox, these would be used to construct 3D plots and to feed our models of neuronal networks.

References:

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