Welcome to University of Washington iGem Team's modeling section. The purpose of this section is to provide architecture, mathematical basis, and further direction for the Vector-Jector project. There will also be some discussion on the motivations and challenges of modeling with respect to both biology and our project. Enjoy!

Vector-Jector

In the beginning of the Vector-Jector project we had a clear view of the hypothetical scenario: yeast, *E. coli*, lactose, and the absence of glucose, leading to horizontal gene transfer. With respect to the modeling efforts, the specificity of the project led to an exhaustive literature search in hopes of creating a comprehensive mathematical model of the system. This process was somewhat successful, but was time consuming and yielded little new perspective. Moreover, this level of focus obscured the creation of a formal architecture and functional model for Vector Jector. Nearing the end of the iGem 2008 run, this lack of cogency became evident, and in response we created, and have frequently revised, Fig. 1 (right). The schema in Fig. 1 has proved to be an invaluable tool, as it provides both a logical checkpoints for modeling rubrics and as it is a structural basis for a generalized Vector-Jector.

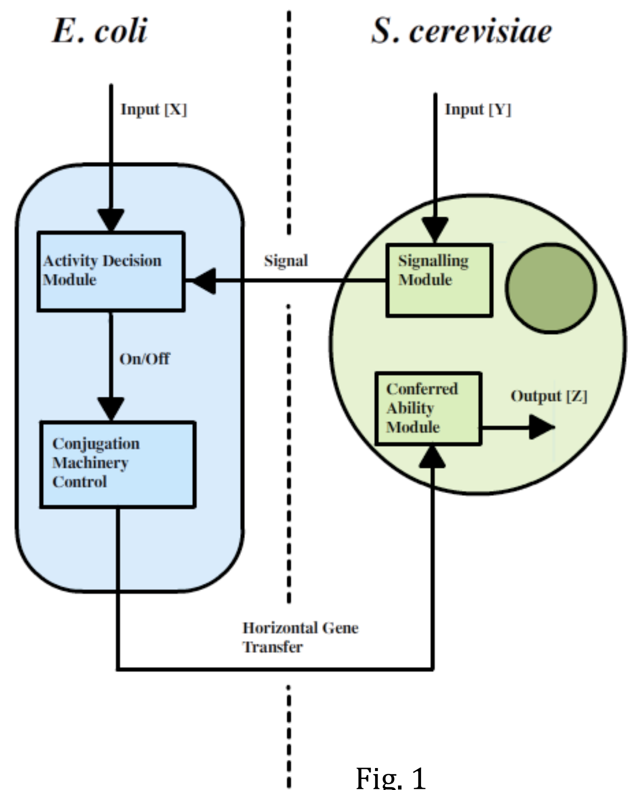

Fig. 1

The purpose of mathematical modeling under this architecture is to verify the desired behavior of the sub-modules, and then perhaps of the entire system. Additionally it could be a tool to 'debug' unexpected behaviors, by providing suggestion to reduce error between the desired and the observed behaviors.

Under the Fig. 1 infrastructure, our implementation for each module is as follows:

- **[1] Signaling Module**: The signaling module has no input, i.e. it is constitutive, and its signal output is a common small molecule: AHL. (A constitutive promoter was the wetlab team's 1st attempt at control over LacI in *S. Cerivisiae*.)

  Input [Y] = [ ] , Output = [AHL]

- **[2] Activity Decision Module**: The activity decision module is a three-input 'and' gate, comprising of two environmental inputs and the *S. cerevisiae* signal; its output is a protein complex that is a common transcription factor.

  Input [X] = [IPTG, Glucose] , Output = [LuxR+AHL complex]

- **[3] Conjugation Machinery Control**: We approached conjugation machinery control in two different methods: methods 1 was controlling the production of a global regulator for conjugation, method 2 was controlling the production of an essential protein in pili construction.

  Input [LuxR+AHL complex] , Output = [Conjugation Activity]

- **[4] Conferred Ability Module**: Our test case for this module was the ability for yeast to produce a certain amino acid, allowing it to grow on deficient media. (Akin to promoter choice in the above signaling module, the Leucine marker was the 1st wetlab implementation)

  Output [Z] = [Leucine]

Models

[1] Signaling Module and [4] Conferred Ability Module:

The models for the Signaling and Conferred Ability modules are fairly basic as they consist of a relatively simple gene networks. In both instances we chose to have production of a gene under a constitutive promoter. The production of AHL includes another synthesis reaction between the enzyme protein LacI and other

ingredient molecules. Hence the differential equation dynamics for these interactions is:

1 RNA Synthesis) $$[R\dot{NA}_{LacI,Leu}] = \beta_{LacI,Leu} - [RNA_{LacI,Leu}](\mu + \gamma_{RNA})$$

2 Protein Translation) $$[Prot\dot{e}in_{LacI,Leu}] = K_{LacI,Leu}[RNA_{LacI,Leu}] - [Protein_{LacI,Leu}](\mu + \gamma_{LacI,Leu})$$

3 AHL Synthesis) $$[A\dot{H}L] = K_{Syn}[LacI] - [AHL](\mu^*_{AHL} + \gamma_{AHL})$$

Where K's and β's represent synthesis rates, μ is a diffusion rate, and γ's represents a degradation rate.

The verification of these models could not be completed due to technical challenges in cloning the AHL creating gene into a yeast vector. However, similar AHL signaling regimes between different bacterial populations have been thoroughly tested. Also note that the AHL concentration presents oversimplified model, as AHL can diffuse in and out of the cell.

[2] Activity Decision Module:

The models for the Activity decision module are certainly the bulk of the modeling efforts. Fig 2. (below) is a diagram of the molecular interactions, which maps the inputs (IPTG, Glucose, and AHL) to the synthesis of the output molecule: LuxR+AHL compound.

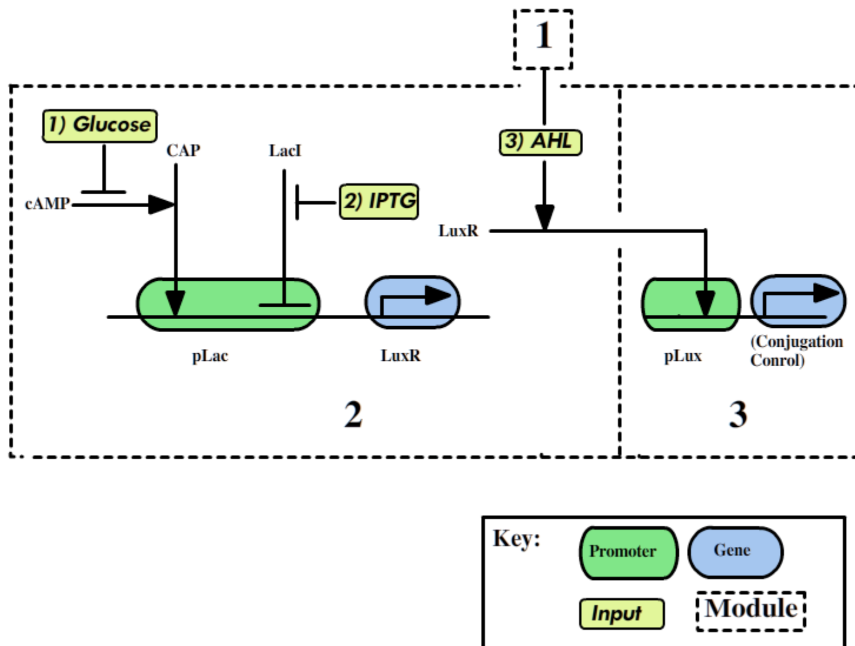

Fig 2.

The diagram does not include a feedback loop between permease activity and IPTG flux. The differential equation models used to describe Fig. 2's system of interactions, including permease interaction, are as follows:

- **Glucose to cAMP synthesis:**
  Wong et. al. 1997 (1)

4 cAMP sythesis) $$[c\dot{AMP}] = V_{cAMP} - [cAMP](\mu + \gamma_{cAMP})$$

Where :

4.1a) $$V_{\mathrm{cAMP}} = \frac{k_{\mathrm{cAMP}}}{\rho}\left(\frac{K_{\mathrm{a,cAMP}}}{[\mathrm{Glu_{ext}}] + K_{\mathrm{a,cAMP}}}\right)$$

4.1b) $$V_{\mathrm{cAMP}} = \frac{k_{\mathrm{cAMP}}}{\rho}\left(\frac{K_{\mathrm{b,cAMP}}}{V_{\mathrm{t,Glu}} + K_{\mathrm{b,cAMP}}}\right)$$

4.2) $$V_{\mathrm{t,Glu}} = k_{\mathrm{t,Glu}}\left(\frac{[\mathrm{Glu_{ext}}]}{[\mathrm{Glu_{ext}}] + K_{\mathrm{t,Glu}}}\right)$$

Recreation of these models using parameters used in (1) was achieved. The difference in equations 4.1a and 4.1b is due to uncertainty as to whether cAMP synthesis is affected by the extracellular glucose level or the transport flux of glucose across the cell membrane. In either case, negative influence on cAMP concentration by glucose concentration was observed *in silico*.

- **IPTG influx:**

IPTG is let in by permease that is encoded off of the lac operon, which is prefaced by the natural lac promoter. Hence permease creation is found in the next section discussing LuxR production.

5 IPTG influx)

$$[IP\dot{T}G] = [Permease]\frac{V_{in}}{K_{I,ext}}\frac{IPTG_{ext} - [IPTG]/K_{iptg}}{(1 + IPTG_{ext}/K_{IPTG,ext} + [IPTG]/K_{in})} - [IPTG](\mu - \gamma_{IPTG})$$

Equation 5 was created with help from our advisors after simulations of published IPTG flux models proved to be instable for all reasonable inputs given the published parameter values. Equation 5 is based off a simple stochastic model and was found to be stable, but its accuracy to experimental data has yet to be verified. Parameters for simulation taken from (4)(5)

- **LuxR and Permease creation:**

LuxR production and Permease production are both driven off of the wild-type lac promoter. The 'and' gate behavior of this promoter is described by Setty et al (2). These equations detail how cAMP and IPTG interact with the lac promoter.

6 LuxR, Permease RNA Synthesis) $$[Lux\dot{R}_{RNA}] = PA^* - [LuxR_{RNA}](\mu - \gamma_{LuxR_{RNA}})$$

7 LuxR, Permease Translation) $$[Lu\dot{x}R] = K_{LuxR}[LuxR_{RNA}] - [LuxR](\mu - \gamma_{LuxR})$$

Where:

8 Promoter Activity Function PA*) $$f = V_1 \frac{1 + V_2\mathcal{A} + V_3\mathcal{R}}{1 + V_4\mathcal{A} + V_5\mathcal{R}}$$

8.1A) $$A = \frac{\frac{[cAMP]}{K_{cAMP}}^n}{1 + \frac{[cAMP]}{K_{cAMP}}^n}$$

8.1R) $$R = \frac{1}{1 + \frac{[IPTG]}{K_{IPTG}}^m}$$

These equations were simulated using parameters from Setty (2). In the Setty experiment exogenous cAMP was added to find cAMP's affect on the lac promoter. In our experiments we were able to use verify behavior of the promoter using the natural ligand glucose. However, the double hill function PA(IPTG,cAMP) has yet to be reconciled to sensitivity of the internal cAMP concentration. Fig 3 below is a recreation of the Setty model, displaying the promoter activity function of the wild type lac promoter.

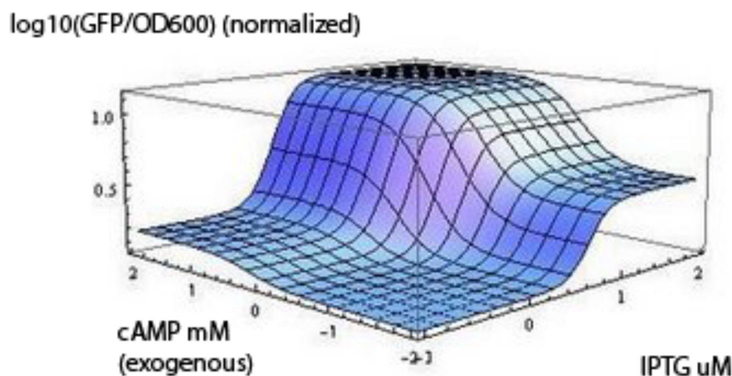

Fig 3.

- **LuxR+AHL Complex Formation:**

The final step of the Activity Decision module is the combination of LuxR and the signaling molecule AHL into the transcription factor LuxR+AHL. Given the setup of our experiment, i.e. fluid incubation of *S. cerevisiae* and *E. coli*, the AHL concentration of the solution is simplified as being uniform throughout the incubation mix. The model and parameters governing LuxR and AHL interaction is taken from Basu et al. (3).

9 LuxR+AHL formation) $$[Lux \dot{+} AHL] = \rho_R [LuxR]^2 [AHL]^2 - [LuxR + AHL](\mu + \gamma_{LuxR+AHL})$$

[3] Conjugation Machinery Control:

The model for the conjugation machinery control was, from the outset of Vector-Jector, going to be a matter of uncertainty. A literature search yielded no previous models for conjugative behavior. Furthermore, serious questions remain about the mechanics of the conjugation process that make the formation of even a simple model difficult. Nevertheless what we could infer was a model for the protein population of conjugation control mechanisms. Adapted from Basu et al (3):

9 Conjugation Control RNA's) $$[RNA_{TrbA,KorA}\dot{]} = \frac{\alpha [LuxR + AHL]^n}{(\theta_R)^n + [LuxR + AHL]^n} - [RNA_{TrbA,KorA}](\mu - \gamma_{TrbA,KorA})$$

10 C.C. Proteins) $$[Protein_{TrbA,KorA}\dot{]} = K_{TrbA,KorA}[RNA_{TrbA,KorA}] - [Protein_{TrbA,KorA}] * (\mu - \gamma_{TrbA,KorA})$$

The effects of manipulating the expression of these conjugation proteins on the conjugation process remain unknown, as many technical problems in cloning hindered us from reaching a testing phase.

The Bigger Picture

The bigger picture of modeling, with respect to our project Vector-Jector, remains somewhat ambiguous. We were able to model subsections of our system from existing models, but these were hardly design considerations when building the system. For a multifaceted project like Vector-Jector, compiling exact models, parameters, and simulations for a multi-stage, multi-organism synthetic system might very well take longer than the project itself; and it is highly unlikely that the sub-models, when consolidated, would predict behavior matching experimental data. The lack of feedback motifs also limits models from being understood in relative terms. However, these are not just problems for Vector-Jector but are considerations for the entire field of synthetic biology.

Future Directions

If we finished the project here are some follow up steps for the modeling process:

- Observe conjugation control protein effects on conjugation efficiency
- Regress parameters for internal cAMP concentration effect on wild type lac promoter
- Gather observational data on conjugation to determine physical characteristics and probabilities.
  - Form simple models given these proabilites

Thanks for reading and hope you have a better understanding of UW iGem's Vector Jector project!

Any questions about the modeling process, or about obtaining the simulations themselves, can be sent to

Tyler Casey
caseyt2@u.washington.edu

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