Models of a Self-Powered Biosensor System for BETX/Salicylate Pollutants

Glasgow iGEM 2007 Team
Toby Friend, Rachael Fulton, Christine Harkness,
Karolis Kidakis, Martina Marbá, Maciej Trybiło

October 23, 2007

1 Introduction

This document describes the design of the models for a biosensor system developed by the Glasgow iGEM 2007 team at the University of Glasgow.

The biosensor that was developed during the project had two versions that differed in the sensing part. In the first version, the $XylR$ protein binds BETX pollutants and the resulting complex works as positive transcription factor on a specific promoter. In the second version, the $DntR$ protein binds salicylate. The overall design of both versions is exactly the same and $XylR/DntR$ is replaced by TF in the models (for "transcription factor"). The pollutants BETX/salicylate are named as s for "signal".

The reporting part of the system are the $PhzM$ and $PhzS$ proteins that catalyse transformation of Phenazine-1-Carboxylic Acid (PCA) compound into pyocyanin (PYO). Pyocyanin is a blue compound thus it provides a visual cue to the experimenter. More interestingly, pyocyanin is also known to have electron mediation ability in a microbial fuel cell [5]. Bacteria closed alone in an anode of a microbial fuel cell have limited ability of producing electric current. A mediator, such as pyocyanin, acts as an oxidant in metabolic reactions of the bacteria and is able to reduce at the anode. In our system increased electrical current induced by the fuel cell indicates existence of a pollutant in the environment.

Two slightly different designs of our system were investigated in the course of the project. The latter is a modification that includes a positive feedback loop in order to enhance system's response to the signal.

2 Towards the Basic Model

The design of the system shown on Figure 1 has to be transformed in order to be effectively modelled.

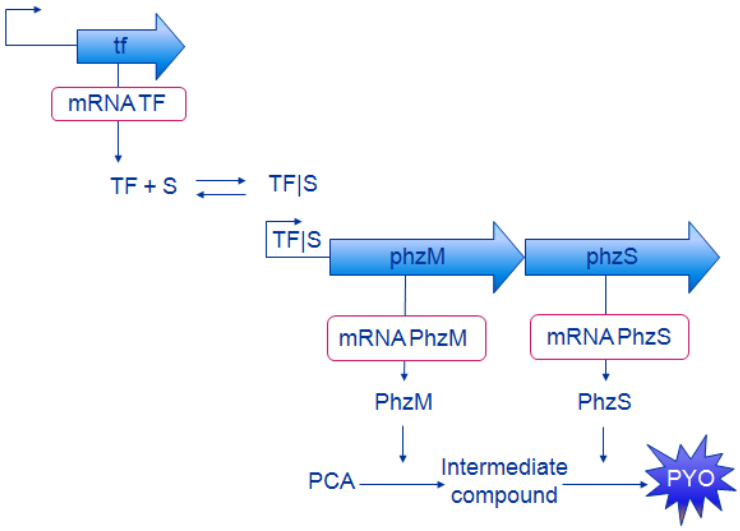

Figure 1: The design of the system. The intermediate compound is 5-methylphenazine-1-carboxylic acid betaine.

In our modelling effort we have omitted the intermediate mRNA production and represented gene expression in one step instead. The resulting model contains less parameters, thus is easier to analyse. Also, there are less parameters that need to be found or estimate. In fact, gene expression rate is often measured disregarding mRNA production.

Production of MPCAB (working name for 5-methylphenazine-1-carboxylic acid betaine - the intermediate compound) has been dropped as well. A study which aimed to characterise this part of the pathway [5] revealed that it is very hard to characterise the $PCA \rightarrow MPCAB$ and $MPCAB \rightarrow PYO$ reactions separately. This is probably due to instability of $MPCAB$. The composite reaction $PCA \rightarrow PYO$ was characterised instead. Therefore, the $MPCAB$ has been completely removed from the model and the $PhzM$ and $PhzS$ proteins have been joined together into $PhzMS$.

The following equations represent the basic model.

$$\dot{TF} = \alpha_{TF} - \delta_{TF} TF - \beta_{TFS} sTF + k_d TFS \tag{1}$$

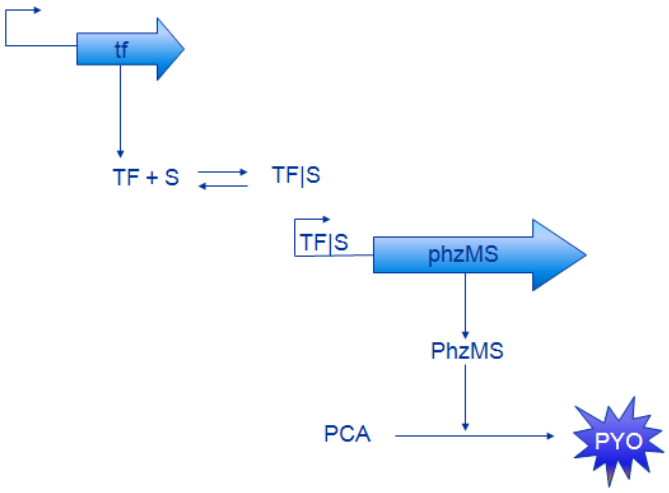

Figure 2: Basic model without mRNA production included.

$$\dot{TFS} = \beta_{TFS} sTF - k_d TFS - \delta_{TFS} TFS \tag{2}$$

$$\dot{PhzMS} = \beta_{PhzMS} \frac{TFS}{\gamma_{PhzMS} + TFS} - \delta_{PhzMS} PhzMS \tag{3}$$

$$\dot{PYO} = \alpha_{PYO} PhzMS - \delta_{PYO} PYO \tag{4}$$

3 Feedback Loop

The second design that was investigated and model created included a positive feedback loop.

The TF is additionally produced when the signal is present. More TF molecules can bind more molecules of S an should increase expression of $PhzMS$. The term $\beta_{TF} \frac{TFS}{\gamma_{TF}+TFS}$ is added to the $\dot{TF}$ equation to represent the additional production of TF.

It is important to note that the basic model and model with feedback loop share many parameters and the parameters that they share have exactly the same meaning in the system. It is crucial for model comparison.

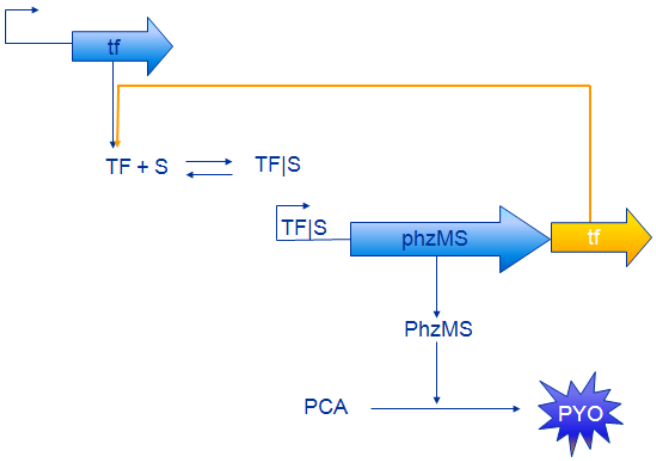

Figure 3: Model M6

$$\dot{TF} = \alpha_{TF} - \delta_{TF}TF - \beta_{TFS}sTF + k_dTFS + \beta_{TF}\frac{TFS}{\gamma_{TF} + TFS} \quad (1)$$

$$\dot{TFS} = \beta_{TFS}sTF - k_dTFS - \delta_{TFS}TFS \quad (2)$$

$$\dot{PhzMS} = \beta_{PhzMS}\frac{TFS}{\gamma_{PhzMS} + TFS} - \delta_{PhzMS}PhzMS \quad (3)$$

$$\dot{PYO} = \alpha_{PYO}PhzMS - \delta_{PYO}PYO \quad (4)$$

Finding parameter values for models is very challenging. There is little information available in the literature mostly because synthetic biology is a novel field and few researchers focus their experiments on measuring rate constants. One who builds

The values that we used in our simulations are presented in Table 1. Some of them come from literature, other have been estimated from "rules of thumb".

No	*name*	*value*	*range*	*comment*
1	α_{TF}			
2	δ_{TF}	3.851e-4 s^{-1}	2.567e-4 - 5.776e-4	Based on 30min half life (range 20-45 for bacterial transcription factors e.g. [6])
3	β_{TFS}	10^6 s^{-1}		Greater than fastest known enzyme
4	γ_{TFS}	4 μM		[1]
5	δ_{TFS}	3.851e-4 s^{-1}	2.567e-4 - 5.776e-4	Based on 30min half life (range 20-45 for bacterial transcription factors e.g. [6])
6	kd			
7	β_{PhzMS}	0.1 s^{-1}		Standard rate for 300 aa bacterial protein
8	γ_{PhzMS}	5 μM	0.1 - 10	From range of DNA-binding constants e.g.[2]
9	δ_{PhzMS}	8.0225e-6 s^{-1}		Based on 24h half life (Bacterial protein norm, e.g. [3])
10	α_{PYO}	1.3 s^{-1}		[5]
11	δ_{PYO}	1.6045e-5 s^{-1}		In human cells [4]. Probably much faster in E. Coli
12	β_{TF}			
13	γ_{TF}			

Table 1: Constants

References

[1] Gordon A. Leonard Sen McSweeney Darcy Birse Irina A. Smirnova, Cyril Dian and Peter Brzezinski. Development of a bacterial biosensor for nitrotoluenes: The crystal structure of the transcriptional regulator dntr. *Journal of Molecular Biology*, 340:405–418, 2004.

[2] Park CH Yang CH Jung KC, Rhee HS. Determination of the dissociation constants for recombinant c-myc, max, and dna complexes: the inhibitory effect of linoleic acid on the dna-binding step. *Biochem Biophys Res Commun*, 334(1):269–75, 2005.

[3] Koch AL Nath K. Protein degradation in escherichia coli. i. measurement of rapidly and slowly decaying components. *J Biol Chem*, 245(11):2889–900, 1970.

[4] Yunxia Q. O'Malley, Maher Y. Abdalla, Michael L. McCormick, Krzysztof J. Reszka, Gerene M. Denning, and Bradley E. Britigan. Subcellular localization of Pseudomonas pyocyanin cytotoxicity in human lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol*, 284(2):L420–430, 2003.

[5] J. Parsons, B. Greenhagen, K. Shi, K. Calabrese, H. Robinson, and J. Ladner. Structural and functional analysis of the pyocyanin biosynthetic protein phzm from pseudomonas aeruginosa. *Biochemistry*, 2007.

[6] Winans SC Zhu J. Autoinducer binding by the quorum-sensing regulator trar increases affinity for target promoters in vitro and decreases trar turnover rates in whole cells. *Proc Natl Acad Sci U S A*, 96(9):4832–7, 1999.