# Modelling techniques and tools to support design and construction in Synthetic Biology

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Environment First Prize & Gold Award

## A Novel System

Our project aimed to design and construct a novel type of power-generating electrochemical biosensor, called ElectrEcoBlu. The output signal is the electro-chemical mediator pyocyanin which enables electrical current to be generated in a microbial fuel cell. ElectrEcoBlu functions as a biosensor for a range of environmental organic pollutants which stimulate the biosensor to produce an electrical power output. The system has the potential to be used for self-powered long term in situ and online monitoring of an electrical output. Our approach exploited a range of state-of-the art modelling techniques to support the design and construction of this novel synthetic biological system.

## Biosensing Fuel Cell

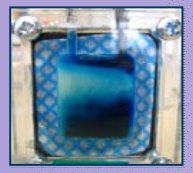


**Figure 2.** A microbial fuel cell.

Organic carcinogenic solvents used in industrial processes and various toxic intermediates from the petrochemical industry can, and unfortunately often do, escape from their containment sites and cause contamination of the surrounding soil and water. For this reason we have developed our biosensor to detect one or several such pollutants including BTEX chemicals, polycyclic aromatic hydrocarbons, intermediates of naphthalene metabolism and phenols.

ElectrEcoBlu could incorporate an unlimited range of detection systems from the Registry of Standard Biological Parts and has the potential to be maintained on-site monitoring conditions constantly.

Pyocyanin, a blue antibiotic, engages in redox reactions and as a result can conduct electricity (4). Its electrochemical properties coupled with its extracellular localisation make it an ideal electron mediator to facilitate power generation in a microbial fuel cell (Figure 2).

## Pollutant Detection

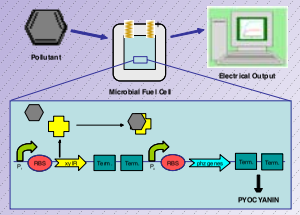



**Figure 3.** Pollutant triggers the bacteria to produce pyocyanin in fuel cells and gives an electrical output.

We cloned pyocyanin genes PhzM and PhzS from *P. aeruginosa* and intend to transfer these with seven gene operon phzABCDEFG into *E. coli* to harvest the redox potential of pyocyanin from a non-pathogenic organism and hence power a microbial fuel cell. We wanted to try several different pollutant-specific sensors to drive transcription of these components.

Benzene, Toluene, Ethylbenzene and Xylenes (BTEX chemicals) are detected by XylR protein. XylR alters in shape when bound to toluene-like chemicals and forms a transcriptional activator which triggers a cascade of metabolic reactions (Figure 3). ElectrEcoBlu will use XylR and related promoters to produce pyocyanin from the phz genes. Using this XylR system we produced luciferase in the presence of toluene (Figure 6).

We chose to try cloning all of these systems into BioBrick format from various sources, from bacterial genomic sequences to artificial historical plasmids already available in the lab.

## Biological results

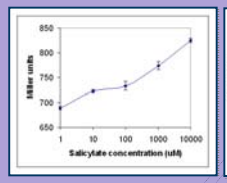



**Figure 4.** The effect of salicylate on LacZ production by *E. coli* containing pQF52 DntR, as measured by the Miller Assay.

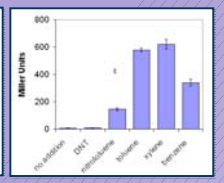
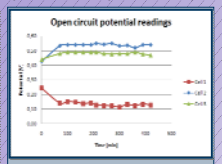



**Figure 5.** The effect of a variety of environmental pollutants on LacZ production by *E. coli* containing pQF52 XylR, as measured by the Miller Assay.


**Figure 6.** Luminescence increased as toluene concentrations increased. The top panel the tubes in the light. The lower panel shows a picture the same 3 tubes exposed for 1/80th of a second.

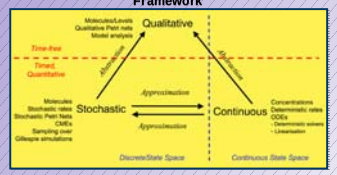



| | | Cell 1 | Cell 2 | Cell 3 |
|---|---|---|---|---|
| Anode | L. Coli | 0ml | 3ml | 0ml |
| | Meth. B. | 0ml | 3ml | 0ml |
| | I.R | 5ml | 3ml | 5ml |
| | Wieler | 0ml | 0ml | 5ml |
| Cathode | Flex. / cm. | 10ml | 10ml | 10ml |

**Figure 7.** Fuel cells were assessed for electrical potential when using methylene blue as an electron mediator.

## Modelling Techniques & Tools

Framework



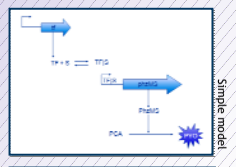

We have used a framework which unifies the qualitative, stochastic and continuous worlds, as a basis for our overall approach to modelling and analysing the biochemical pathways.

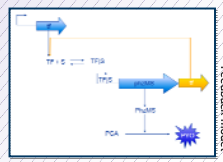
## The Models

Biologists and the modellers together developed a simple model that describes the biosensor system.

After investigating many different topologies to enhance the response of the system positive feedback was considered by the team to be the most promising.

Various computational techniques were used to analyse these models.




## Ordinary Differential Equations (ODE)

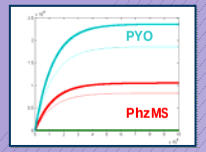
$$TF = \alpha_{TF} - \delta_{TF}TF - \beta_{TFS}TF + k_dTFS$$

$$\boxed{+\beta_{PhzS}\frac{TFS}{\gamma_{TF}+TFS}}$$

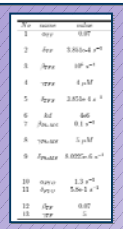
$$TFS = \beta_{TFS}TF - k_dTFS - \delta_{TFS}TFS$$

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

$$PYO = \alpha_{PYO}PhzMS - \delta_{PYO}PYO$$

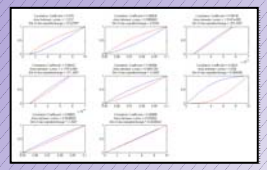



ODEs were developed to represent the above models. Highlighted in orange is the addition of the positive feedback. The simulation plot generated using the parameters on the table beside is shown in the figure on the top right. Feedback model in bold - yields higher response.



## Multi Parametric Sensitivity Analysis

We have used the MPSA and its modified version - comparative MPSA in order to discover significant parameters within a model, and parameters that are significant to the difference between the models with positive feedback and without.



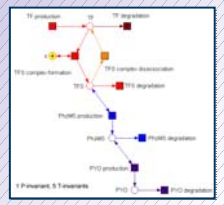
The plot shows comparative analysis we have done on the most uncertain parameters. The plot in the third column, second row shows the most significant parameter - γ_TF. This information can be used to drive parameter estimation - the most sensitive parameters are the ones that are most important to estimate finely.

## Petri Nets

Petri nets provide users with an intuitive and easy-to-understand graphical representation of systems.

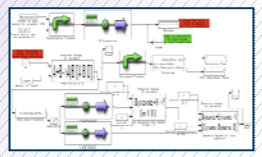
We started off with the construction of qualitative Petri-net models, in order to perform analysis without kinetic parameters.
We then constructed quantitative Continuous Petri Nets which have an ODE-based semantics.

Tools: Snoopy for simulations and Charlie for qualitative analysis.



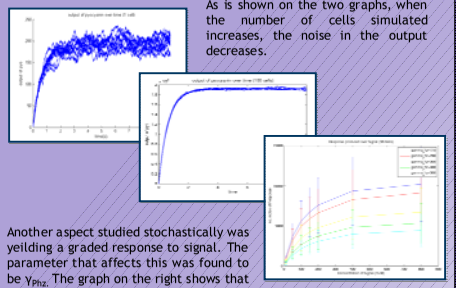
## BioBrick Graphical Environment (Simulink)

We have developed an environment for component-based for dynamic modelling of biological systems at Brick (Gene) level.



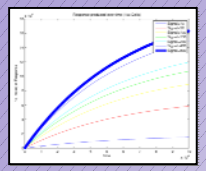
## Stochastic Modelling

Modelling bilogical systems in terms of ODE's is often not enough as cells are intrinsically stochastic. This can lead to significant statistical fluctuations in reaction rates and, the system's behaviour can differ from that predicted by a deterministic model.
A stochastic model for the biosensor was constructed using a computational approach called Gillespie algorithm and simulated over different number of cells.



As is shown on the two graphs, when the number of cells simulated increases, the noise in the output decreases.

Another aspect studied stochastically was yeilding a graded response to signal. The parameter that affects this was found to be $\gamma_{Phz}$. The graph on the right shows that a higher value of $\gamma_{Phz}$ gives a more graded response of the output to signal.



The graph to the left shows mean output of pyocyanin over 100 runs. Each line represents different value of signal. As expected when the concentration of the signal increases the output saturates at a later time thus, giving a graded response to the input signal.

## References

(1) Rogers, K. R., 2005 Recent advances in biosensor techniques for environmental monitoring *Analytica Chimica Acta* 568:222-231

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(3) Ron, E. Z. 2007 Biosensing environmental pollution *Current Opinion in Biotechnology* 18:252-258

(4) Parsons, J. F., Greenhagen, B. T., Shi, K., Calabrese, K., Robinson, H., Ladner, J. E. 2007 Structural and Functional Analysis of the Pyocyanin Biosynthetic Protein PhzM from *Pseudomonas aeruginosa* *Biochemistry* 46:1821-1828

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## Tools & Resources

- ElectrEcoBlu wiki pages including technical reports, models and software http://parts.mit.edu/igem07/index.php/Glasgow
- Snoopy and Charlie: www-dssz.informatik.tu-cottbus.de
- MatLab: www.mathworks.com
- iGEM project code including Minicap Sensitivity Analysis Program: parts.mit.edu/igem07/index.php/Glasgow/
- BioNessie (University of Glasgow): www.bionessie.org

## Thanks & Sponsors

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