Talk Outline

• Background
• System design
• Novel reporter system
• Established modelling techniques
• Cutting-edge modelling
The Problem

Phenolic compounds

Polycyclic aromatic hydrocarbons (PAH)

BTEX compounds
Objectives

1. Design modular sensor construct
2. Create the construct
3. Test the system
4. Development into a machine
5. Model and predict outcomes!
Why a Biosensor?

• Lab-based monitoring
• Skilled workforce
• Expensive!
What is a Biosensor?

- Biosensors include a transcriptional activator coupled to a reporter
What is a Biosensor?

- Biosensors include a transcriptional activator coupled to a reporter
What is a Biosensor?

- Biosensors include a transcriptional activator coupled to a reporter

- **XylR**

- **toluene**

- Luciferase gene

- **luciferin**

- **luminescence**

- **operator / promoter**

- **Luciferase gene**
Our Construct Design

Constitutive promoter | RBS | Transcriptional activator | Double terminator BBa_B0015

Responsive promoter BBa_J61101 | Reporter gene | Double terminator BBa_B0015
Objectives

• 1: Design modular sensor construct
  – Switch on reporter in presence of pollutants

• 2: Create the construct

• 3: Test the system

• 4: Development into a machine

• 5: Model and predict outcomes!
Our Solution

Phenolic compounds
DmpR - phenols

Polycyclic aromatic hydrocarbons (PAH)
DntR - PAHs

BTEX compounds
XylR - toluene
Our Construct Design

Constitutive promoter

Responsive promoter

Transcriptional activator

Double terminator BBa_B0015

RBS

RBS BBa_J61101

Reporter gene

Double terminator BBa_B0015
Objectives

1: Design modular sensor construct
   - Switch on reporter in presence of pollutants

2: Create the construct
   - Use 3 different sensors to express luciferase or LacZ

3: Test the system

4: Development into a machine

5: Model and predict outcomes!
Testing The System
Testing The System

DntR - inducible LacZ

![Graph showing the relationship between [PAH metabolite] (µM) and Miller Units.](image)
Testing The System

DntR - inducible LacZ

XylR - inducible luciferase

![Graph and images of vials with different levels of PAH metabolite concentrations](image-url)
Objectives

• 1: Design sensor/reporter construct
  – Switch on reporter in presence of pollutants
• 2: Create the construct
  – Use 3 different sensors to express luciferase or LacZ
• 3: Test the system
  – PAH-metabolite and xylene sensors successful
• 4: Development into a machine
• 5: Model and predict outcomes!
Unique Reporter System

• Conventional biosensors use conventional reporter genes
  – e.g. LacZ, GFP, luciferase…

• Lengthy and expensive procedures

• Need a novel idea!
Microbial Fuel Cells

- Clean, renewable & autonomous
- Electrons from metabolism harvested at anode
- Versatile, long-lasting, varied carbon sources
- Advantage over conventional power sources
Microbial Fuel Cells
Pyocyanin

• From pathogenic *Pseudomonas aeruginosa*
Pyocyanin

• Phz genes – 7 gene operon, pseudomonad specific

• PhzM and PhzS – *P. aeruginosa* specific

Biosynthesis of pyocyanin
Our Constructs
Our Constructs

- Constitutive promoter
- RBS
- Inducible transcription factor
- Double terminator
Our Constructs

Constitutive promoter  RBS  Inducible transcription factor  Double terminator

Target promoter  RBS  PhzM coding region  RBS  PhzS coding region  Double terminator
Pollutant → Microbial Fuel Cell → Electrical Output

- xylR
- RBS
- Term.
- Term.
- P
- Pr
- RBS
- xyIR
- Term.
- Term.
- Pu
- RBS
- phz genes
- Term.
- Term.
- PYOCYANIN

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Objectives

1: Design sensor/reporter construct
   - Switch on reporter in presence of pollutants

2: Create the construct
   - Use 3 different sensors to express luciferase or LacZ

3: Test the system
   - PAH-metabolite and xylene sensors successful

4: Development into a machine
   - Use *Pseudomonas aeruginosa* to power a fuel cell
     which generates a remote signal sent to base station

5: Model and predict outcomes!
Computational Modelling of the Biosensor

- Aims
  - Guide biologists for the better design of synthetic networks
  - Use different computational approaches to model and analyze the systems
    - Simple biosensor
    - Positive feedback within the biosensor
  - Test and Validate the hypothesis proposed by the biologists
The Model

TF: Dntr or Xylr
S: signal
TF|S: complex

mRNA TF

TF + S ↔ TF|S

TF|S

mRNA PhzM
PhzM
PCA
Intermediate compound
PYO

mRNA PhzS
PhzS
The Model

- Merge transcription and translation

**TF**: Dntr or Xylr  
**S**: signal  
**TF|S**: complex
The Model

- Merge transcription and translation

TF: Dntr or Xylr
S: signal
TF|S: complex
The Model

- Merge transcription and translation
- Merge phzM with phzS (Parsons 2007)

TF: Dntr or Xylr
S: signal
TF|S: complex

TF + S ↔ TF|S

TF|S → phzM → PhzM → PCA → Intermediate compound → PYO

phzS → PhzS
The Model

- Merge transcription and translation
- Merge phzM with phzS (Parsons 2007)

**TF:** Dntr or Xylr

**S:** signal

**TF|S:** complex

**PCA** → **PYO**
Feedback Loop

TF: Dntr or Xylr
S: signal
TF|S: complex

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Feedback Loop

TF: Dntr or Xylr
S: signal
TF|S: complex
Modelling framework

- Time-free
  - Molecules/Levels
  - Qualitative Petri nets
  - Model analysis

- Timed, Quantitative
  - Molecules
  - Stochastic rates
  - Stochastic Petri Nets
  - CMEs
  - Sampling over Gillespie simulations

Approximation

Discrete State Space

Continuous State Space

Approximation

- Concentrations
  - Deterministic rates
  - ODEs
  - Deterministic solvers
  - Linearisation
Modelling framework

- Qualitative
  - Molecules/Levels
  - Qualitative Petri nets
  - Model analysis

- Stochastic
  - Molecules
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  - Stochastic Petri Nets
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- Continuous
  - Concentrations
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- Time-free
- Timed, Quantitative

Approximation

Discrete State Space

Continuous State Space
Qualitative Petri-Net Modelling & Analysis

- Graphical representation--Snoopy

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Qualitative Petri-Net Modelling & Analysis

- Graphical representation--Snoopy
- Qualitative analysis Charlie
  - T invariants (cyclic behavior in pink)
  - P invariants
  - (constant amount of output)
Qualitative Petri-Net Modelling & Analysis

- Graphical representation--Snoopy
- Qualitative analysis Charlie
  - T invariants (cyclic behavior in pink)
  - P invariants
  - (constant amount of output)
- Quantitative Analysis by continuous Petri Net
  - ODE Simulation
Modelling framework

- **Qualitative**
  - Molecules/Levels
  - Qualitative Petri nets
  - Model analysis

- **Stochastic**
  - Molecules
  - Stochastic rates
  - Stochastic Petri Nets
  - CMEs
  - Sampling over Gillespie simulations

- **Continuous**
  - Concentrations
  - Deterministic rates
  - ODEs
  - Deterministic solvers
  - Linearisation

Time-free

Timed, Quantitative
Parameters

- Literature search
- Experts’ knowledge

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Ordinary Differential Equations

\[
\begin{align*}
\dot{TF} &= \alpha_{TF} - \delta_{TF}TF - \beta_{TFS}S + k_dTFS + \beta_{TF} \frac{TFS}{\gamma_{TF} + TFS} \\
\dot{TFS} &= \beta_{TFS}STF - k_dTFS - \delta_{TFS}TFS \\
\dot{PhzMS} &= \beta_{PhzMS} \frac{TFS}{\gamma_{PhzMS} + TFS} - \delta_{PhzMS}PhzMS \\
\dot{PYO} &= \alpha_{PYO}PhzMS - \delta_{PYO}PYO
\end{align*}
\]
Ordinary Differential Equations

\[
\begin{align*}
\dot{T}F &= \alpha_{TF} - \delta_{TF}TF - \beta_{TFS}SF + k_dTFS \\
&\quad + \beta_{TF} \frac{TFS}{\gamma_{TF} + TFS} \\
\dot{T}FS &= \beta_{TFS}SF - k_dTFS - \delta_{TFS}TFS \\
\dot{PhzMS} &= \beta_{PhzMS} \frac{TFS}{\gamma_{PhzMS} + TFS} - \delta_{PhzMS}PhzMS \\
P\dot{YO} &= \alpha_{PYO}PhzMS - \delta_{PYO}PYO
\end{align*}
\]
Ordinary Differential Equations

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\dot{TF} &= \alpha_{TF} - \delta_{TF}TF - \beta_{TFS}S + k_d TFS \\
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PYO &= \alpha_{PYO} PhzMS - \delta_{PYO} PYO
\end{align*}
\]

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Ordinary Differential Equations

\[
\begin{align*}
\dot{T}F &= \alpha_{TF} - \delta_{TF}TF - \beta_{TFS}TF + k_dTFS + \beta_{TF}\frac{TFS}{\gamma_{TF} + TFS} \\
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\dot{PYO} &= \alpha_{PYO}PhzMS - \delta_{PYO}PYO
\end{align*}
\]
Ordinary Differential Equations

\[ \dot{T}F = \alpha_{TF} - \delta_{TF}TF - \beta_{TFS}S + k_{d}TFS + \beta_{TF} \frac{TFS}{\gamma_{TF} + TFS} \]  
(1)

\[ \dot{T}FS = \beta_{TFS}S - k_{d}TFS - \delta_{TFS}TFS \]  
(2)

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

\[ PYO = \alpha_{PYO}PhzMS - \delta_{PYO}PYO \]  
(4)
Parameters

- Literature search
- Experts’ knowledge

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Model Parameter Refinement

- Modified MPSA
Modelling framework

- Molecules/Levels
- Qualitative Petri nets
- Model analysis

- Time-free

- Timed, Quantitative

- Approximation
- Abstraction

- Stochastic
- Continuous

- Discrete State Space
- Continuous State Space

- Concentrations
- Deterministic rates
- ODEs
- Deterministic solvers
- Linearisation

- Sampling over Gillespie simulations
- Stochastic Petri Nets
- CMEs
- Stochastic rates

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Advantages and disadvantages of stochastic modelling

• Living systems are intrinsically stochastic due to low numbers of molecules that participate in reactions
• Gives a better prediction of the model on a cellular level
• Allows random variation in one or more inputs over time
• Slow simulation time
Chemical Master Equations

A set of linear, autonomous ODE’s, one ODE for each possible state of the system. The system may be written:

- $\Phi \rightarrow TF$ - production of TF
- $TF \rightarrow \Phi$ - degradation of TF
- $TF+S \rightarrow TFS$ - association of TFS
- $TFS \rightarrow TF+S$ - dissociation of TFS
- $TFS \rightarrow \Phi$ - degradation of TFS
- $\Phi \rightarrow PhzMS$ - production of PhzMS
- $PhzMS \rightarrow \Phi$ - degradation of PhzMS
- $PhzMS \rightarrow PYO$ - production of pyocyanin
- $PYO \rightarrow \Phi$ - degradation of pyocyanin
### Propensity Functions

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<td>$\phi \rightarrow TF$</td>
<td>$\alpha = c(1)$</td>
<td>$a(1) = c(1)$</td>
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<tr>
<td>$TF \rightarrow \phi$</td>
<td>$\delta_{TF} = c(2)$</td>
<td>$a(2) = c(2) \times X(1)$</td>
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<tr>
<td>$TF + S \rightarrow TFS$</td>
<td>$K1 \times S = c(3)$</td>
<td>$a(3) = c(3) \times X(1)$</td>
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<tr>
<td>$TFS \rightarrow TF + S$</td>
<td>$Km1 = c(4)$</td>
<td>$a(4) = c(4) \times X(2)$</td>
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<td>$\delta_{TFS} = c(5)$</td>
<td>$a(5) = c(5) \times X(2)$</td>
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<td>$a(6) = c(6)$</td>
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<tr>
<td>$P3 \rightarrow \phi$</td>
<td>$\tilde{\delta}_{P3} = c(7)$</td>
<td>$a(7) = c(7) \times X(3)$</td>
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<td>$a(9) = c(9) \times X(4)$</td>
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In the end...

Our Contributions:

- standard SBML models of the systems
- new biobricks with mathematical description
- Practical comparison of modelling approaches – qualitative, continuous, stochastic, based on sound theoretical framework
- Tools to support synthetic biology (Code available):
  - Minicap: multi-parametric sensitivity analysis of dynamic systems
  - Simulink environment
Objectives

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   - Switch on reporter in presence of pollutants

2: Create the construct
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4: Development into a machine
   - Use *Pseudomonas aeruginosa* to power a fuel cell
     which generates a remote signal sent to base station

5: Model and predict outcomes!
Our Constructs So Far...

- Native promoter
- Native RBS
- XylR responsive promoter
- RBS BBa_J61101
- XylR BBa_J52008
- Renilla Luciferase BBa_J52008
- Double Terminator BBa_B0015
Our Constructs So Far…

Native promoter  Native RBS  XylR  Double Terminator BBa_B0015

XylR responsive promoter  RBS BBa_J61101  Renilla Luciferase BBa_J52008  Double Terminator BBa_B0015

IRES  XylR
Our Constructs So Far...

Native promoter

Native RBS

XylR

Double Terminator BBa_B0015

XylR responsive promoter

RBS

Renilla Luciferase BBa_J52008

IRES

XylR

Double Terminator BBa_B0015
## Registry Contributions

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<td>Xylene-sensitive promoter</td>
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<td>BBa_I723023</td>
<td>Xylene-inducible luciferase</td>
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<td>BBa_I723031</td>
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Students

• Toby Friend
• Rachael Fulton
• Christine Harkness
• Mai-Britt Jensen
• Karolis Kidykas
• Martina Marbà
• Lynsey McLeay
• Christine Merrick
• Maija Paakkunainen
• Scott Ramsay
• Maciej Trybiło

Instructors

• David Forehand
• David Gilbert
• Gary Gray
• Xu Gu
• Raya Khanin
• David Leader
• Susan Rosser
• Emma Travis
• Gabriela Kalna
Thank You!