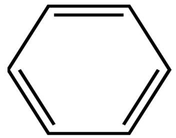
GLASGOW ELECTREGOBLU

iGEM 2007

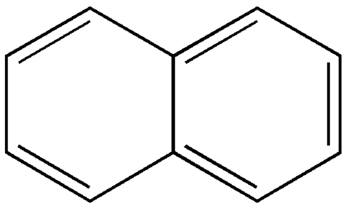
Talk Outline

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

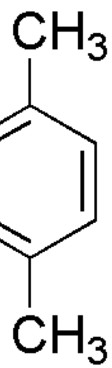
The Problem



Phenolic compounds



Polycyclic aromatic hydrocarbons (PAH)



BTEX compounds

Objectives

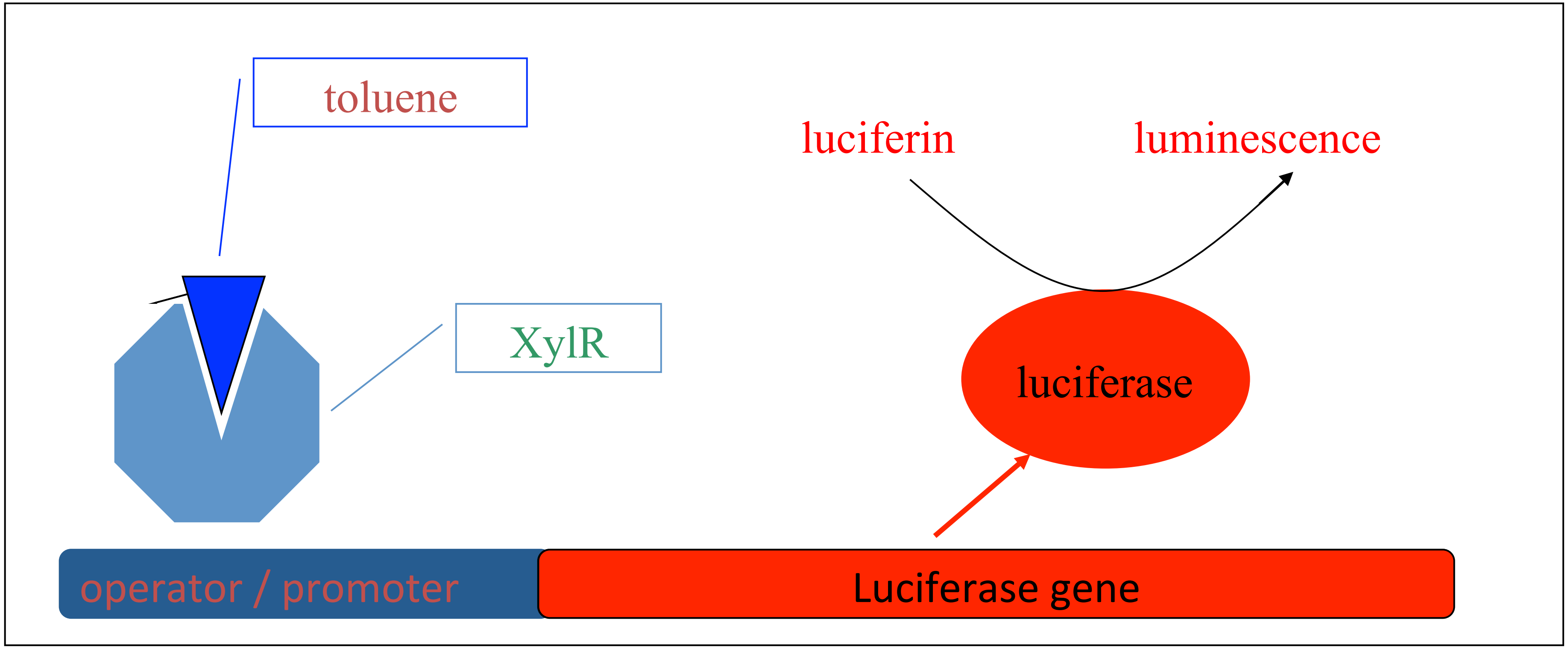
- 1: Design modular sensor construct
- 2: Create the construct
- 3: Test the system
- 4: Development into a machine
- 5: Model and predict outcomes!

Why a Biosensor?

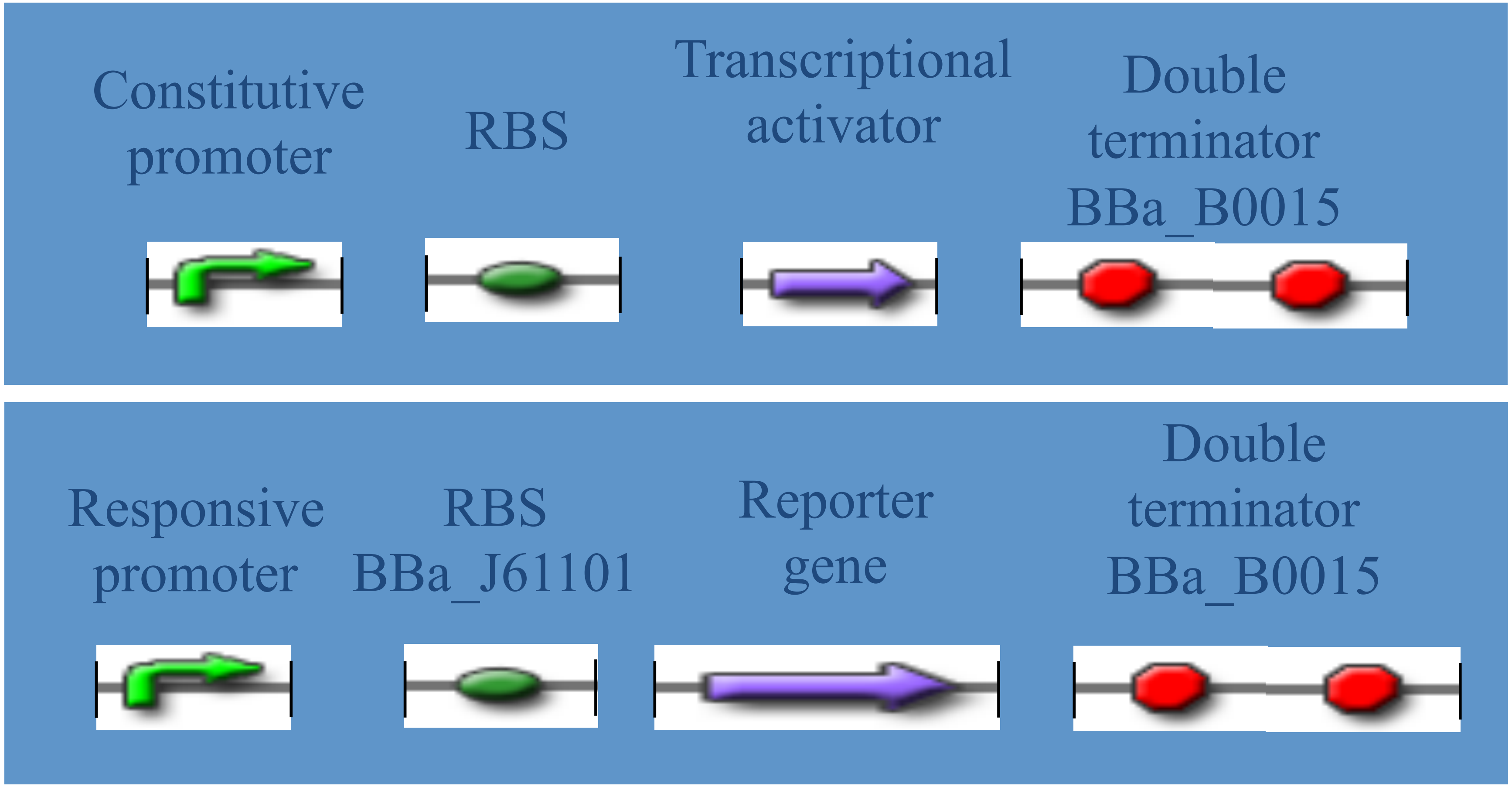
- Lab-based monitoring
- Skilled workforce
- Expensive!

What is a Biosensor?

- Biosensors include a transcriptional activator coupled to a reporter



Our Construct Design



Objectives

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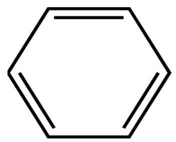
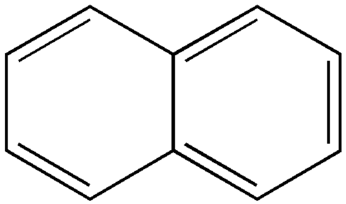
Our Solution



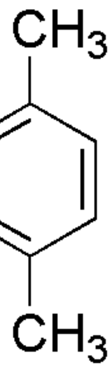
Phenolic compounds

DmpR - phenols



Polycyclic aromatic hydrocarbons (PAH)

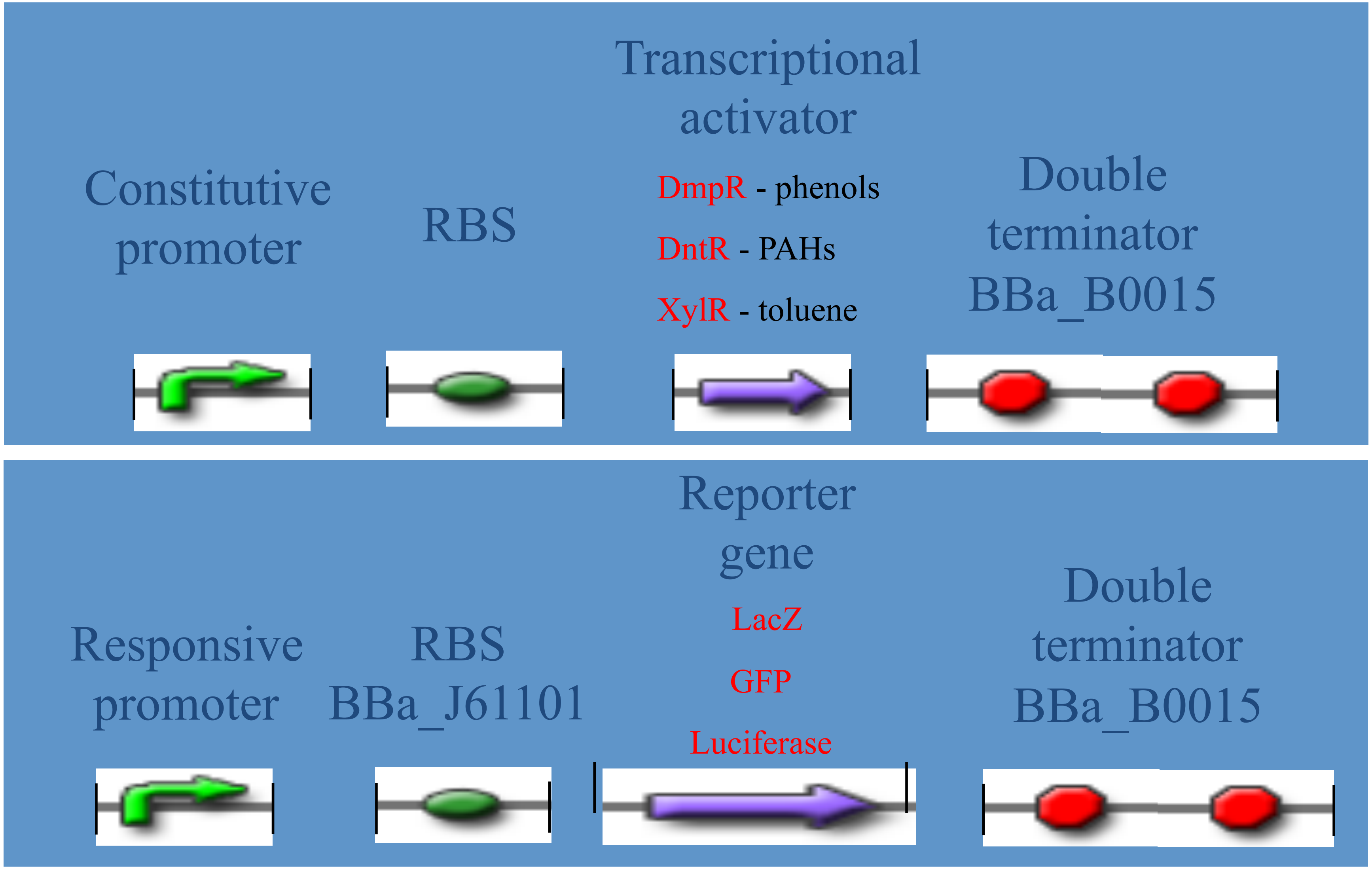
DntR - PAHs



BTEX compounds

XylR - toluene

Our Construct Design



Objectives

- 1: Design modular sensor construct
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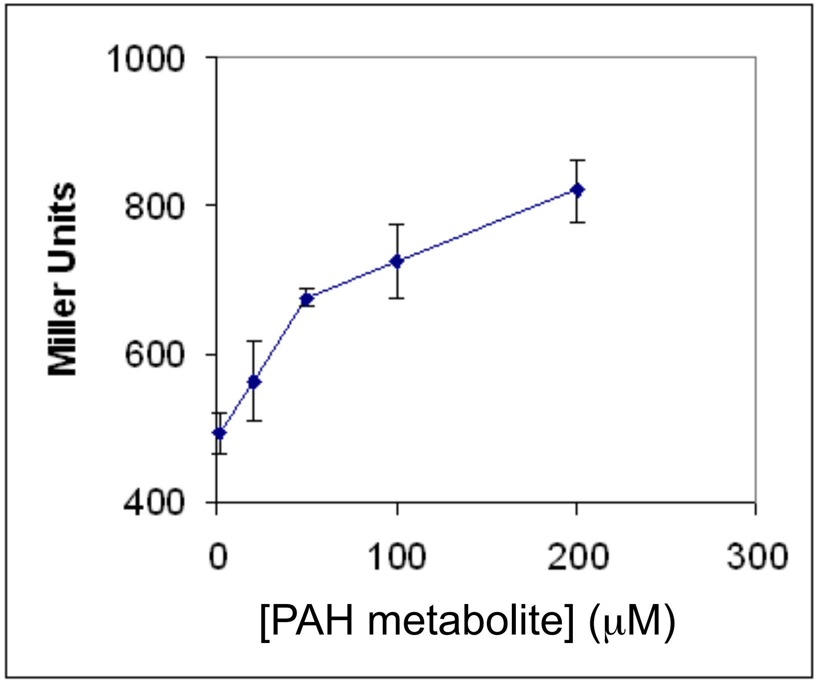
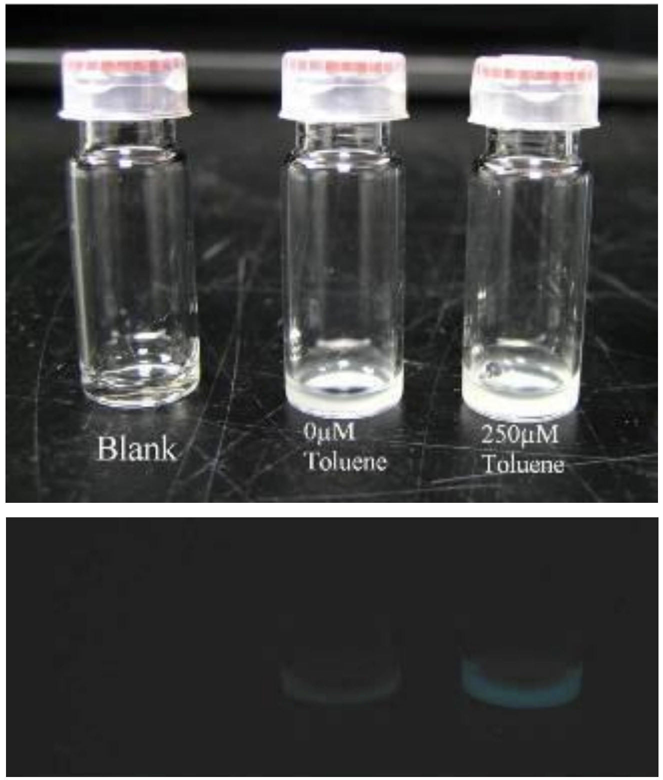
Testing The System

DntR - inducible LacZ



XylR - inducible luciferase



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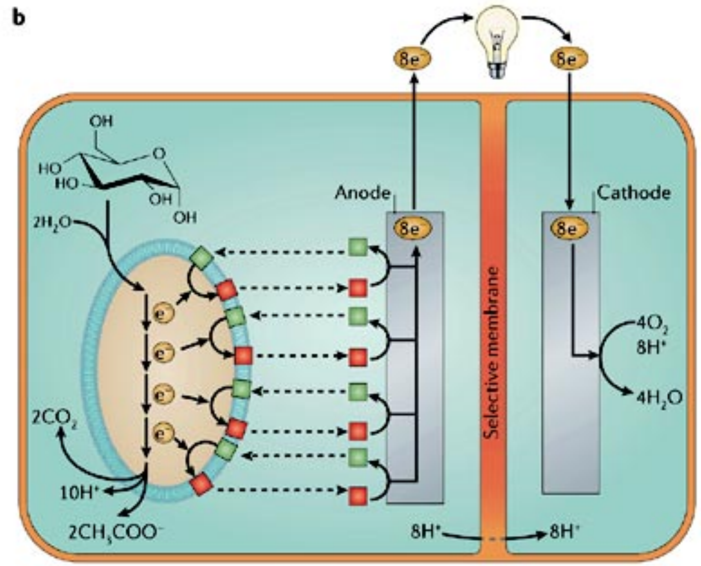
Unique Reporter System

- 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

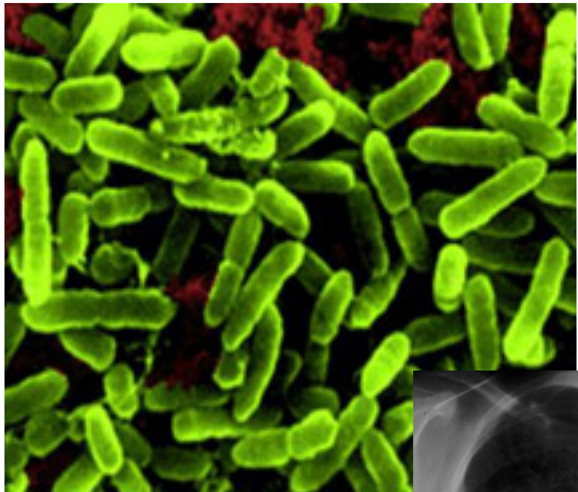
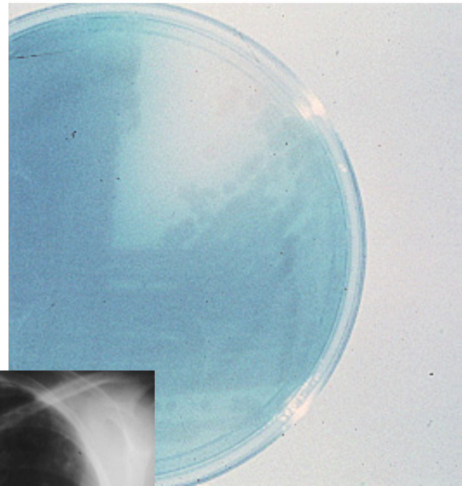
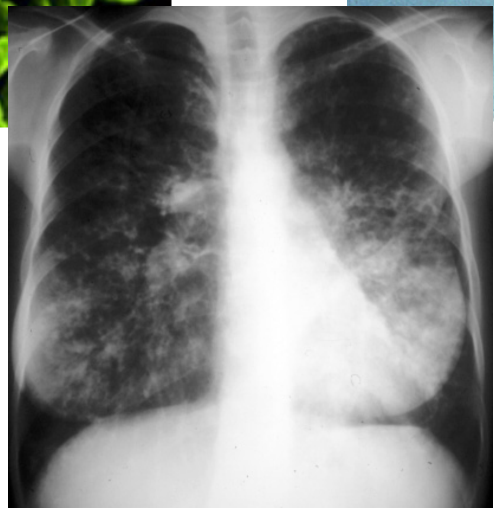
Microbial Fuel Cells





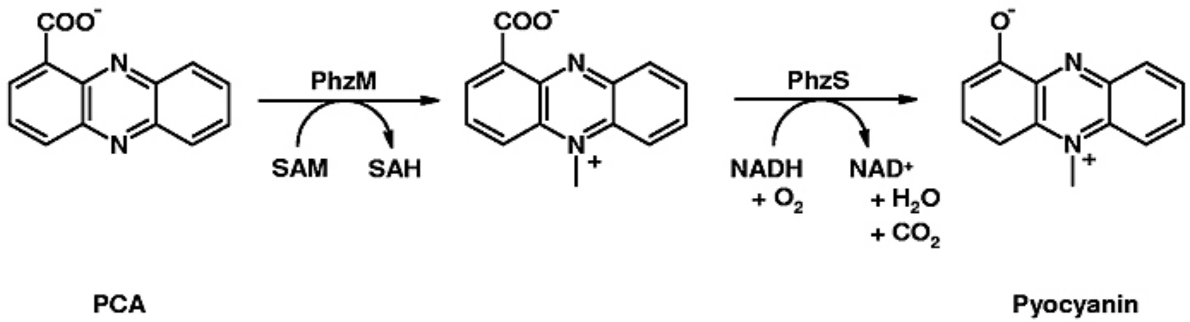

Pyocyanin

- From pathogenic *Pseudomonas aeruginosa*

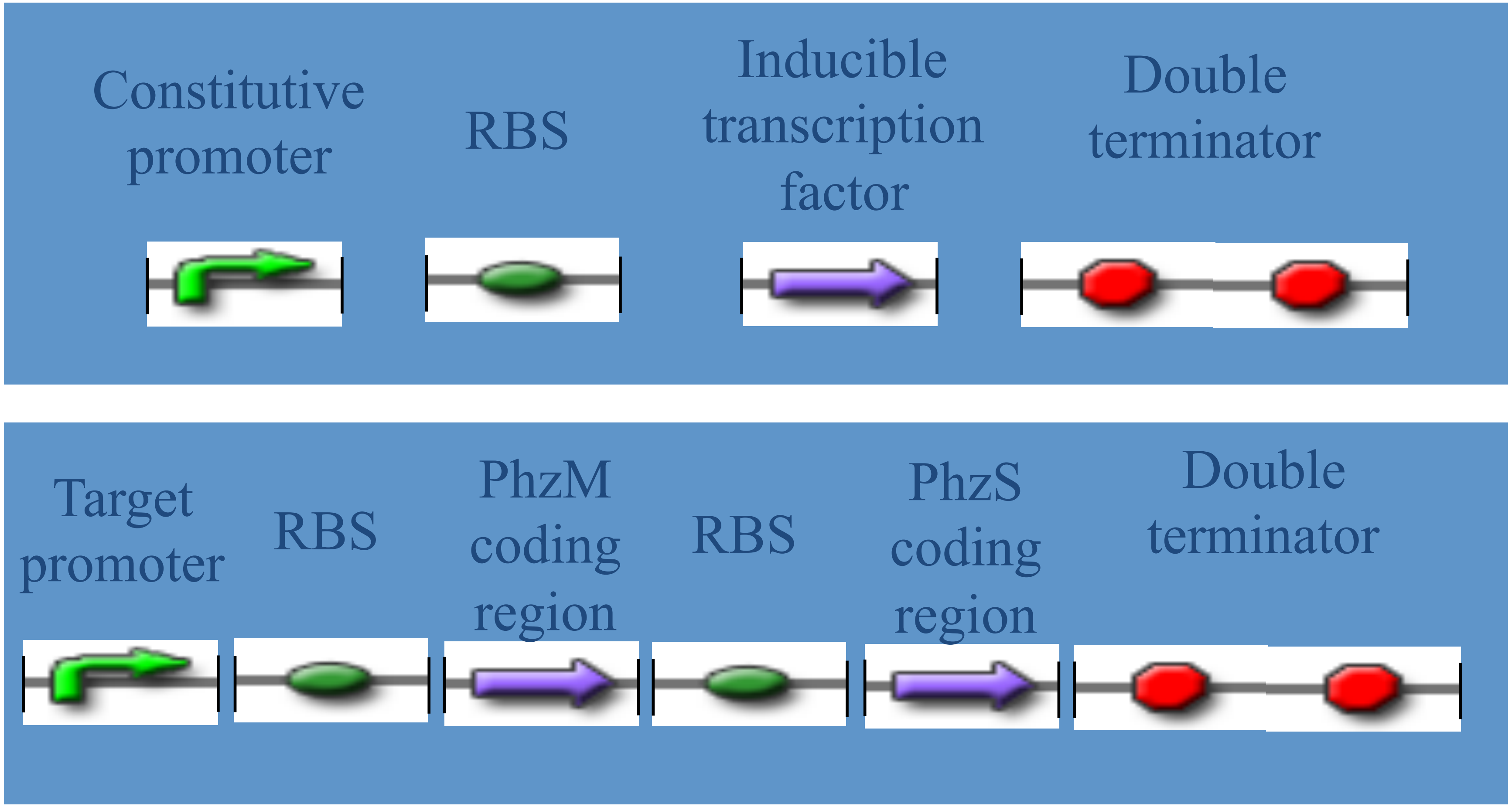
Pyocyanin

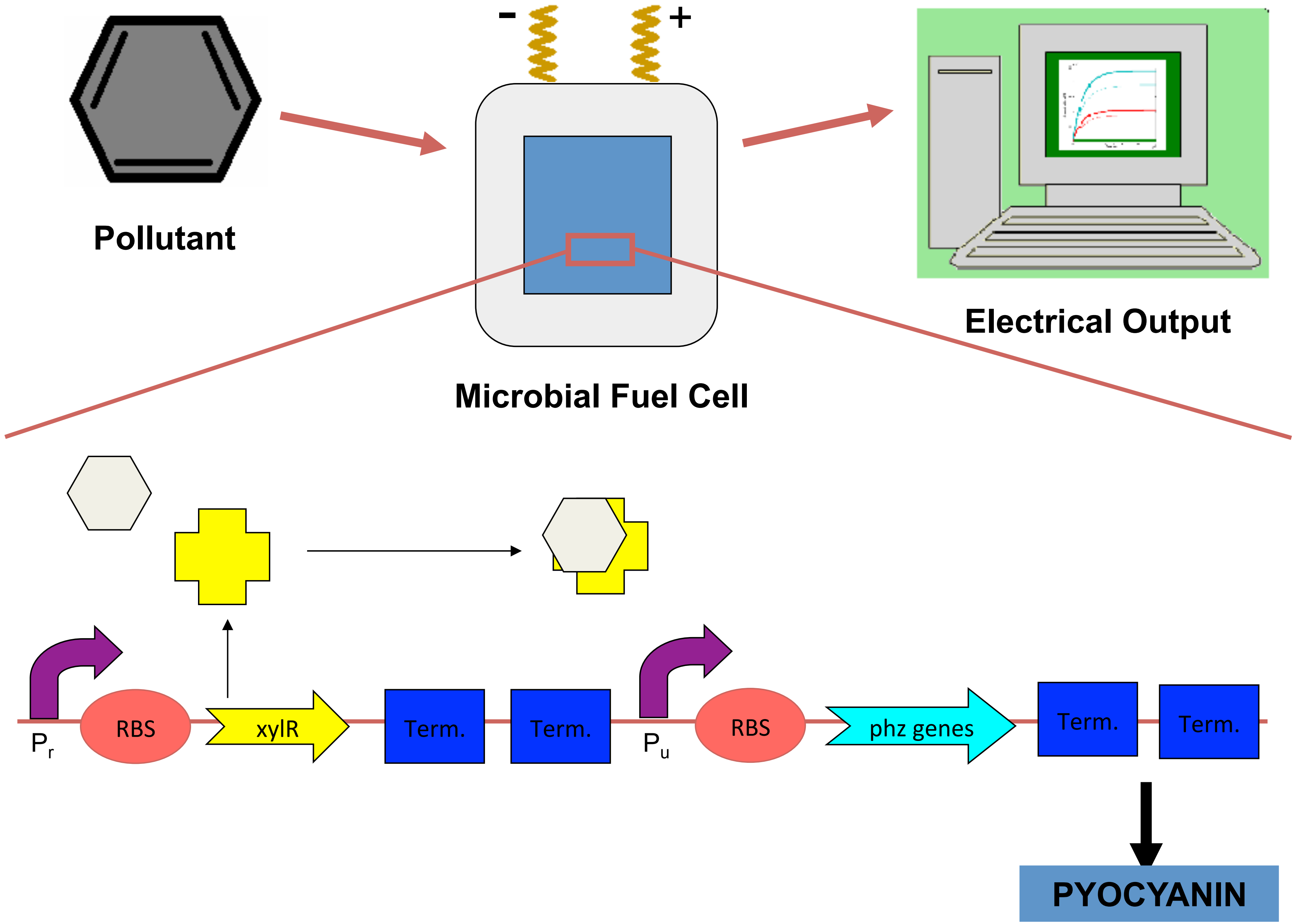
- Phz genes – 7 gene operon, pseudomonad specific
- PhzM and PhzS – *P. aeruginosa* specific



Biosynthesis of pyocyanin

Our Constructs





Pollutant
Microbial Fuel Cell
Electrical Output
P_r
RBS
xylR
Term.
Term.
P_u
RBS
phz genes
Term.
Term.
PYOCYANIN

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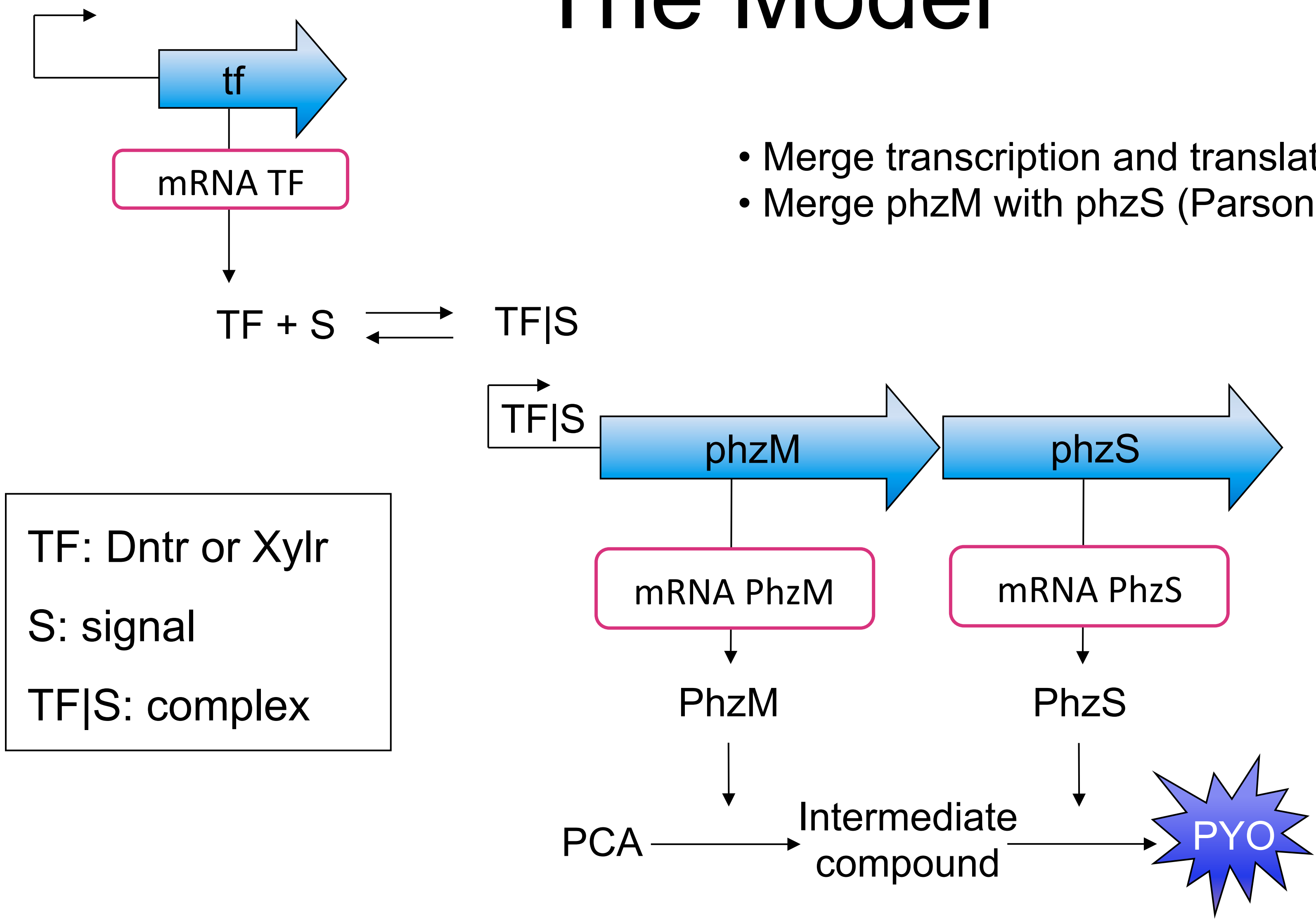
Wetlab - Drylab

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
    - o Simple biosensor
    - o Positive feedback within the biosensor
  - Test and Validate the hypothesis proposed by the biologists

The Model

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



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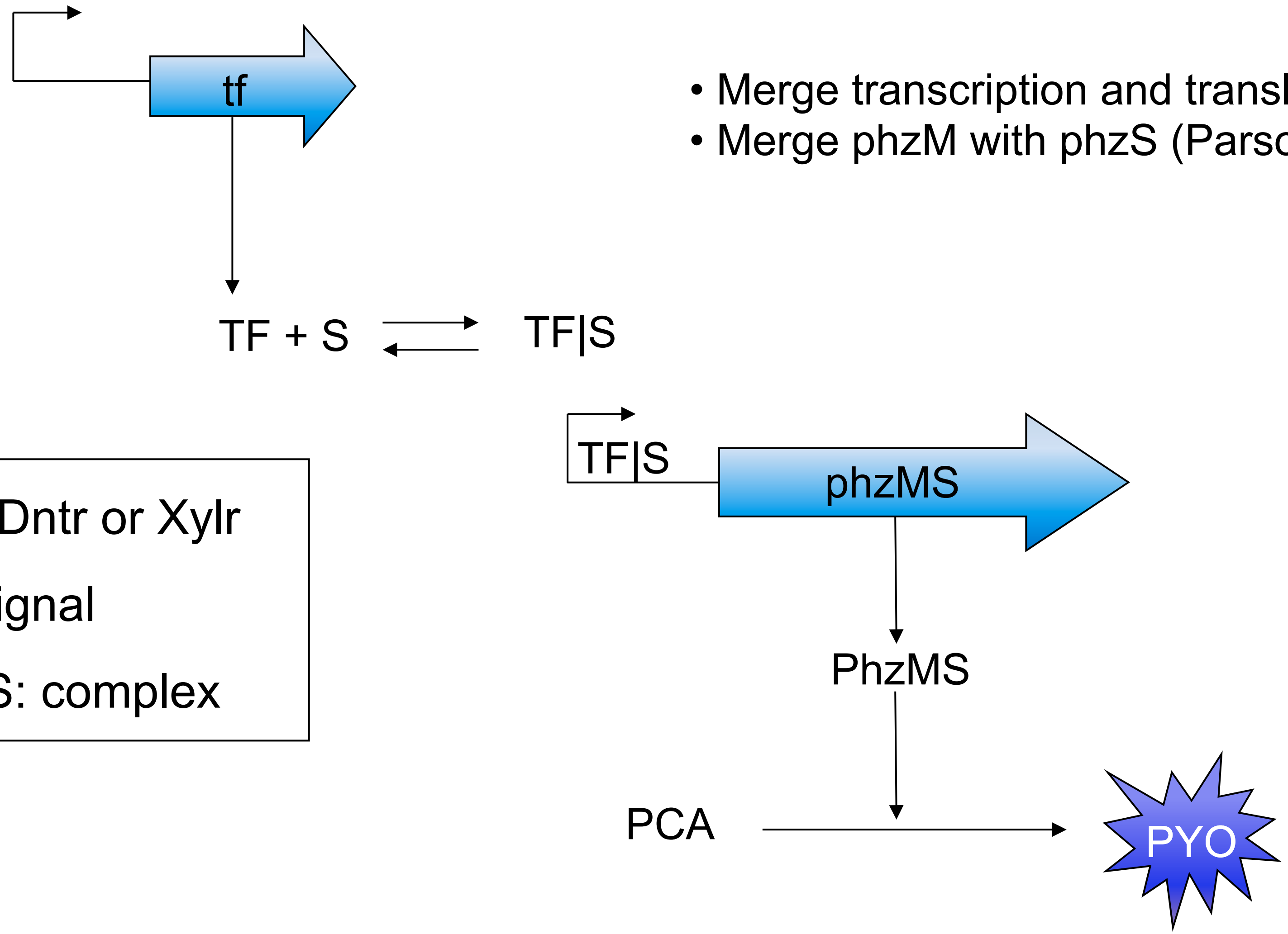
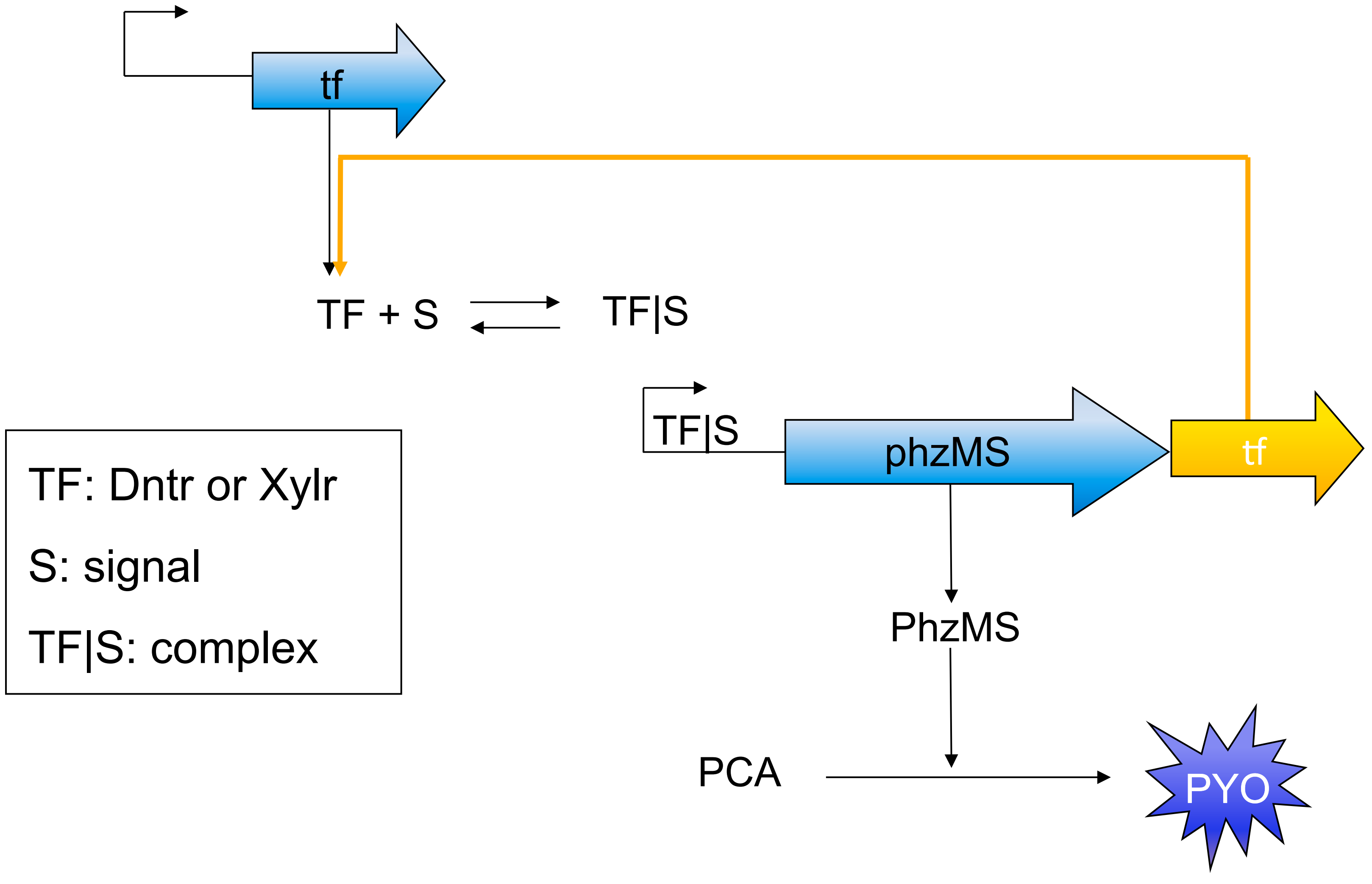


TF: Dntr or Xylr

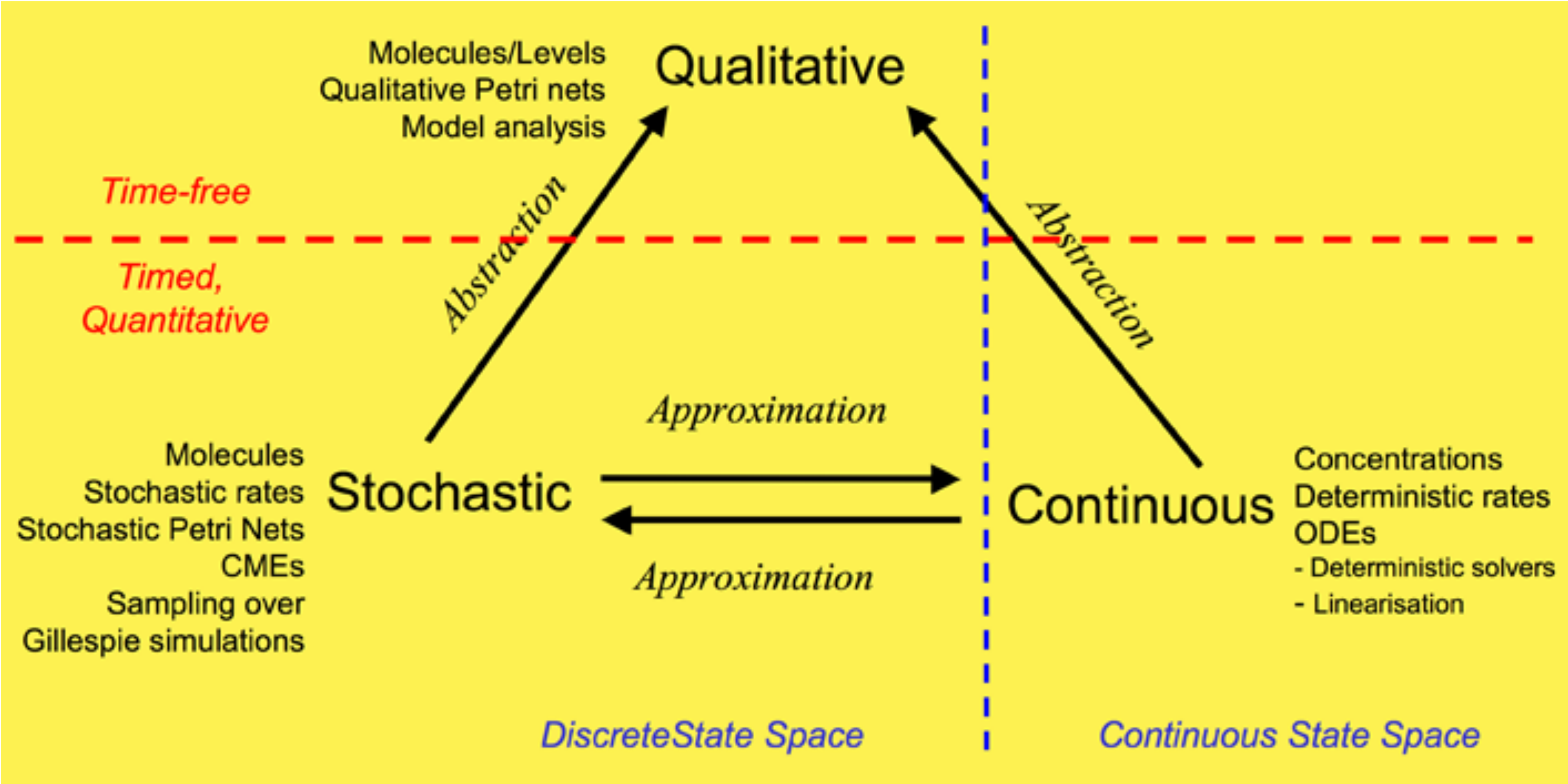
S: signal

TF|S: complex

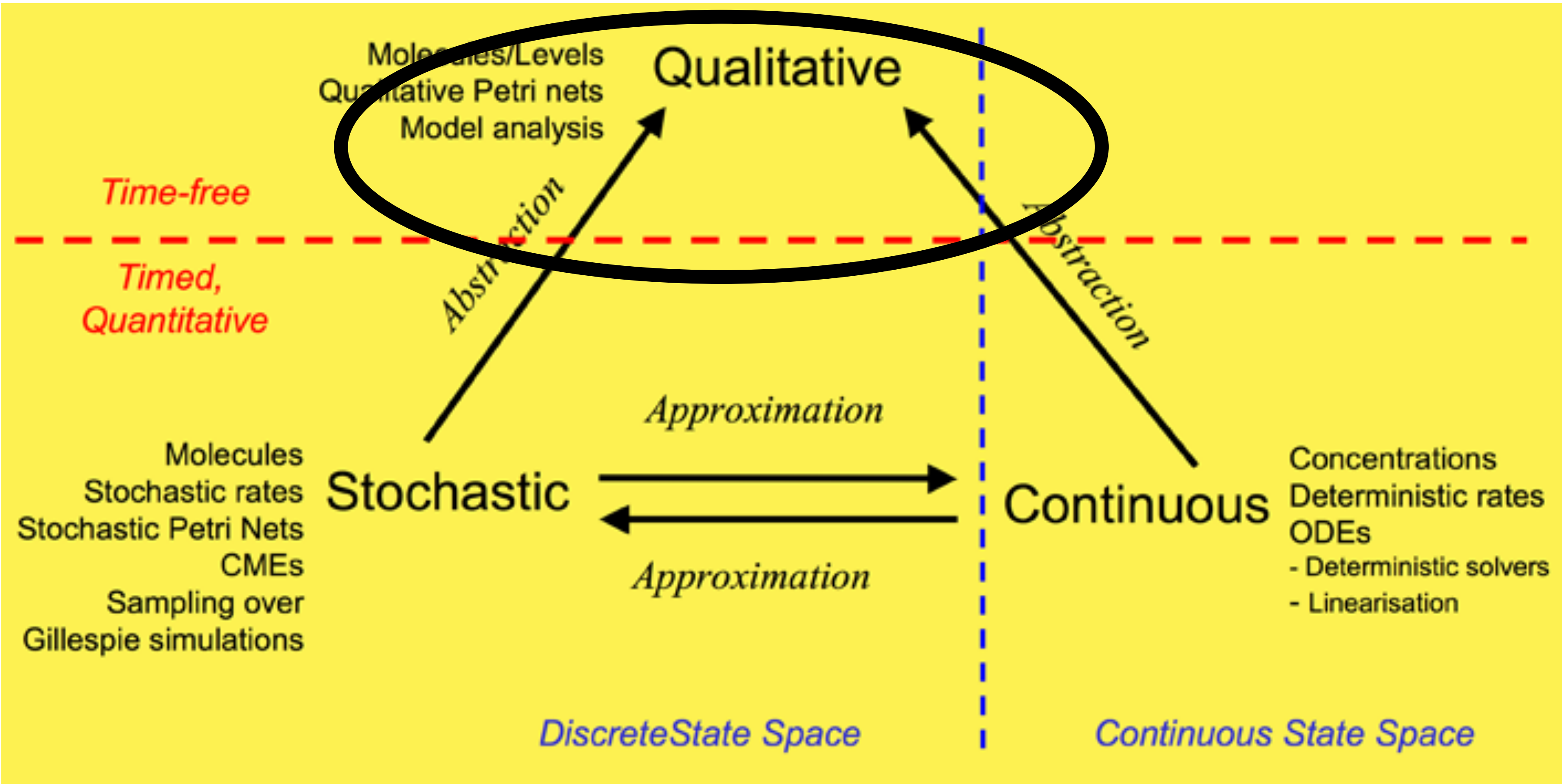
Feedback Loop



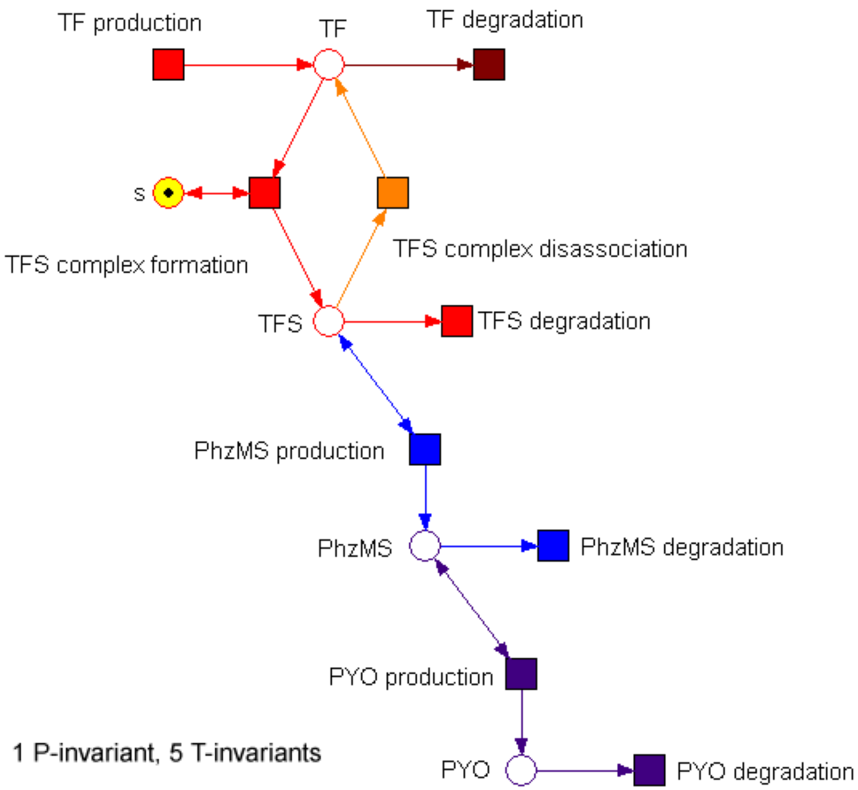
Modelling framework



Modelling framework



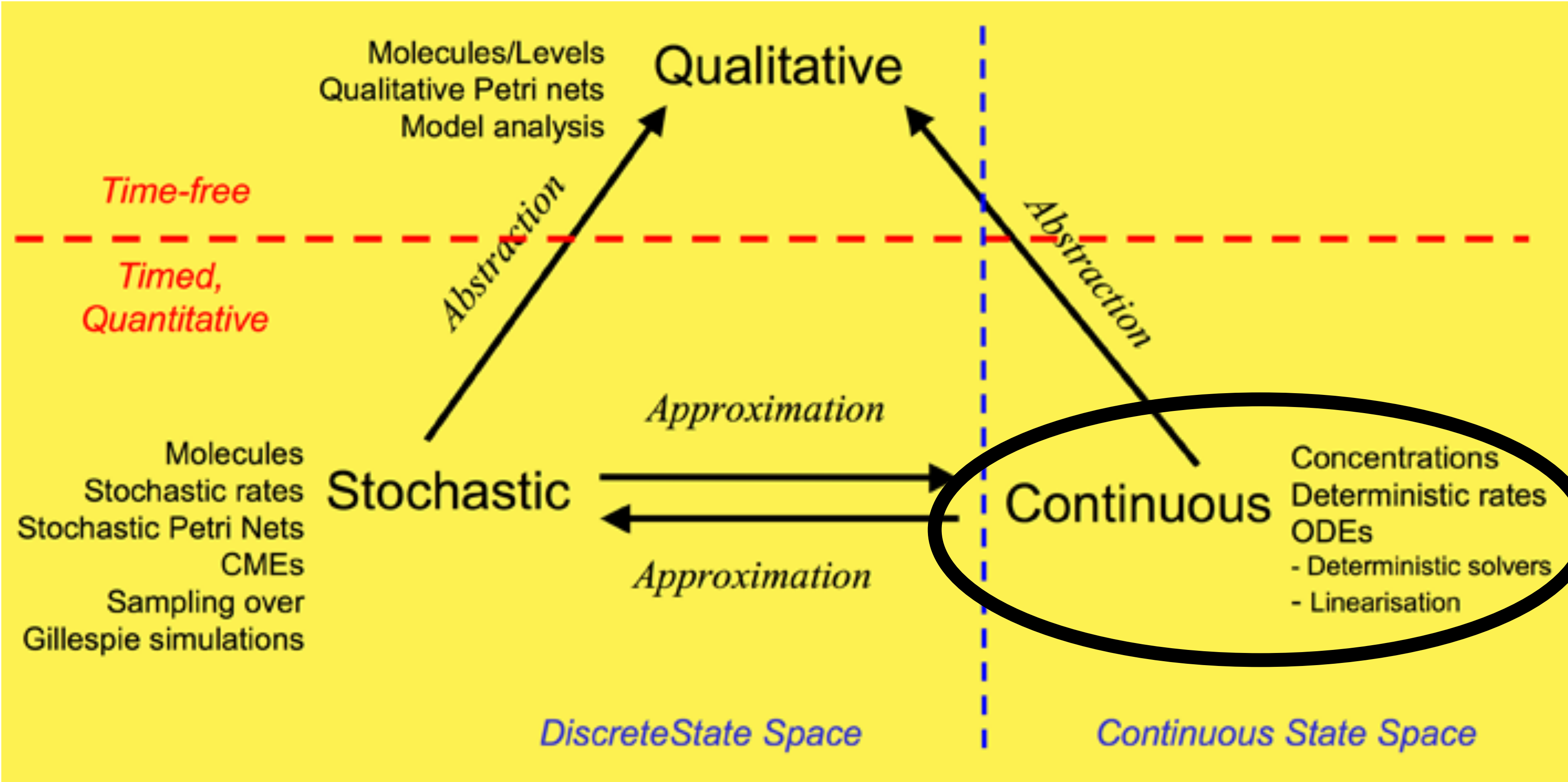
Qualitative Petri-Net
Modelling & Analysis



- Graphical representation--Snoopy
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



Parameters

- Literature search
- Experts' knowledge

No	$name$	$value$	$range$
1	α_{TF}	0.07	0.05 - 0.1
2	δ_{TF}	3.851e-4 s^{-1}	2.567e-4 - 5.776e-4
3	β_{TFS}	10^6 s^{-1}	
4	γ_{TFS}	4 μM	
5	δ_{TFS}	3.851e-4 s^{-1}	2.567e-4 - 5.776e-4
6	kd	4e6	
7	β_{PhzMS}	0.1 s^{-1}	
8	γ_{PhzMS}	5 μM	0.1 - 10
9	δ_{PhzMS}	8.0225e-6 s^{-1}	
10	α_{PYO}	1.3 s^{-1}	
11	δ_{PYO}	5.8e-1 s^{-1}	
12	β_{TF}	0.07	0.05 - 0.1
13	γ_{TF}	5	0.1 - 10

Ordinary Differential Equations

SBML Available!

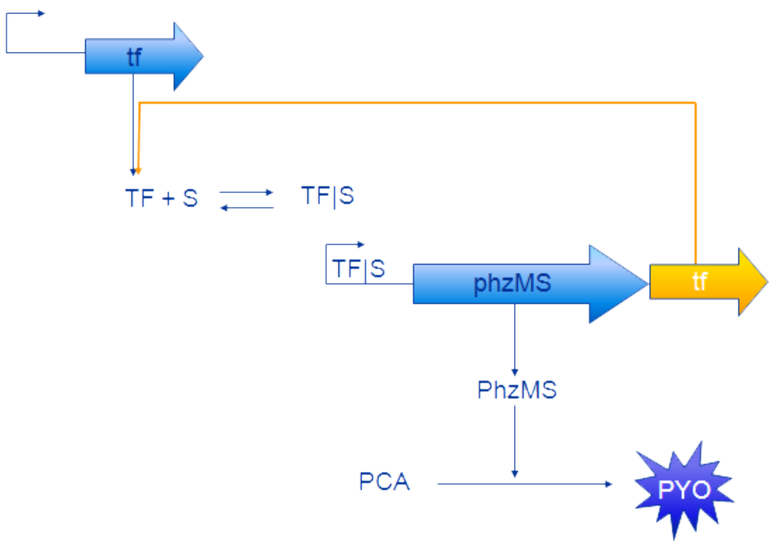
Created in BioNessie

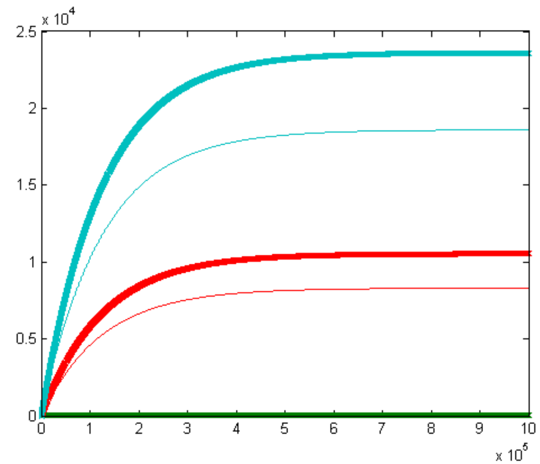
$$\dot{TF} = \alpha_{TF} - \delta_{TF}TF - \beta_{TFS}sTF + k_dTFS + \beta_{TF}\frac{TFS}{\gamma_{TF} + TFS} \qquad (1)$$

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

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

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





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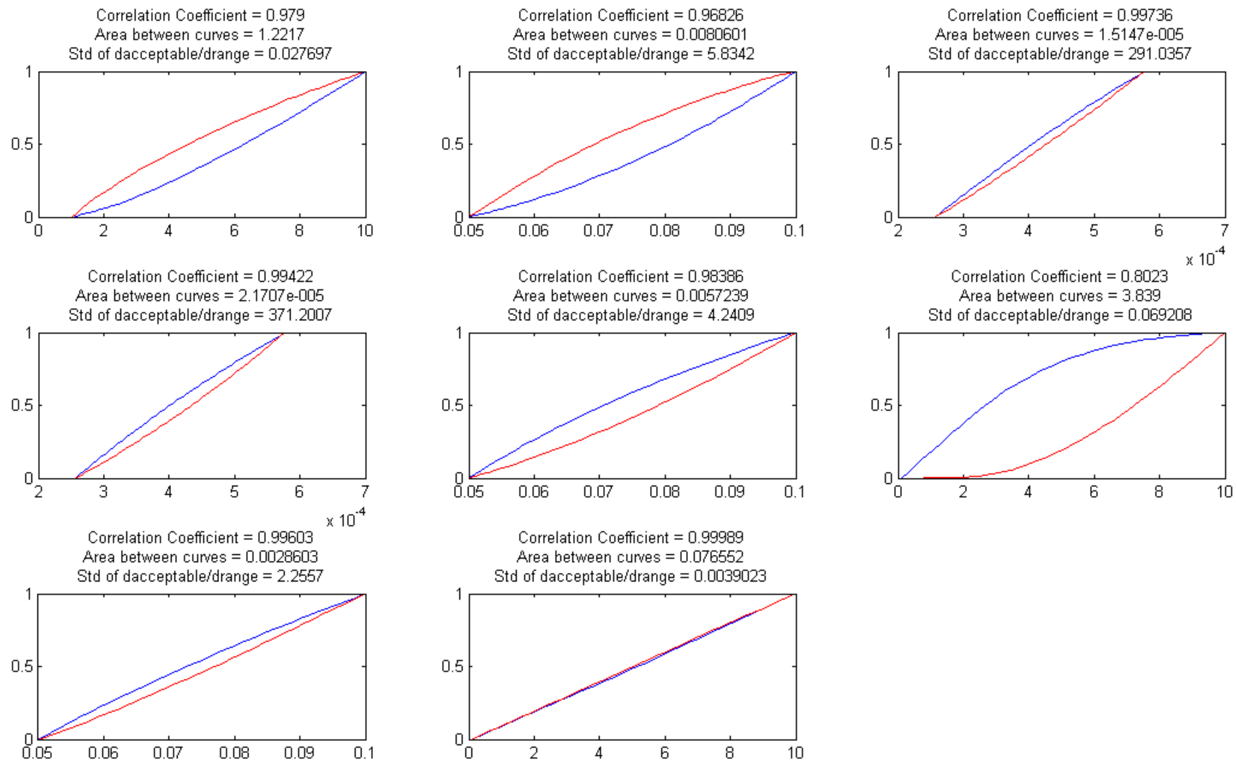
Model Parameter Refinement

- Modified MPSA



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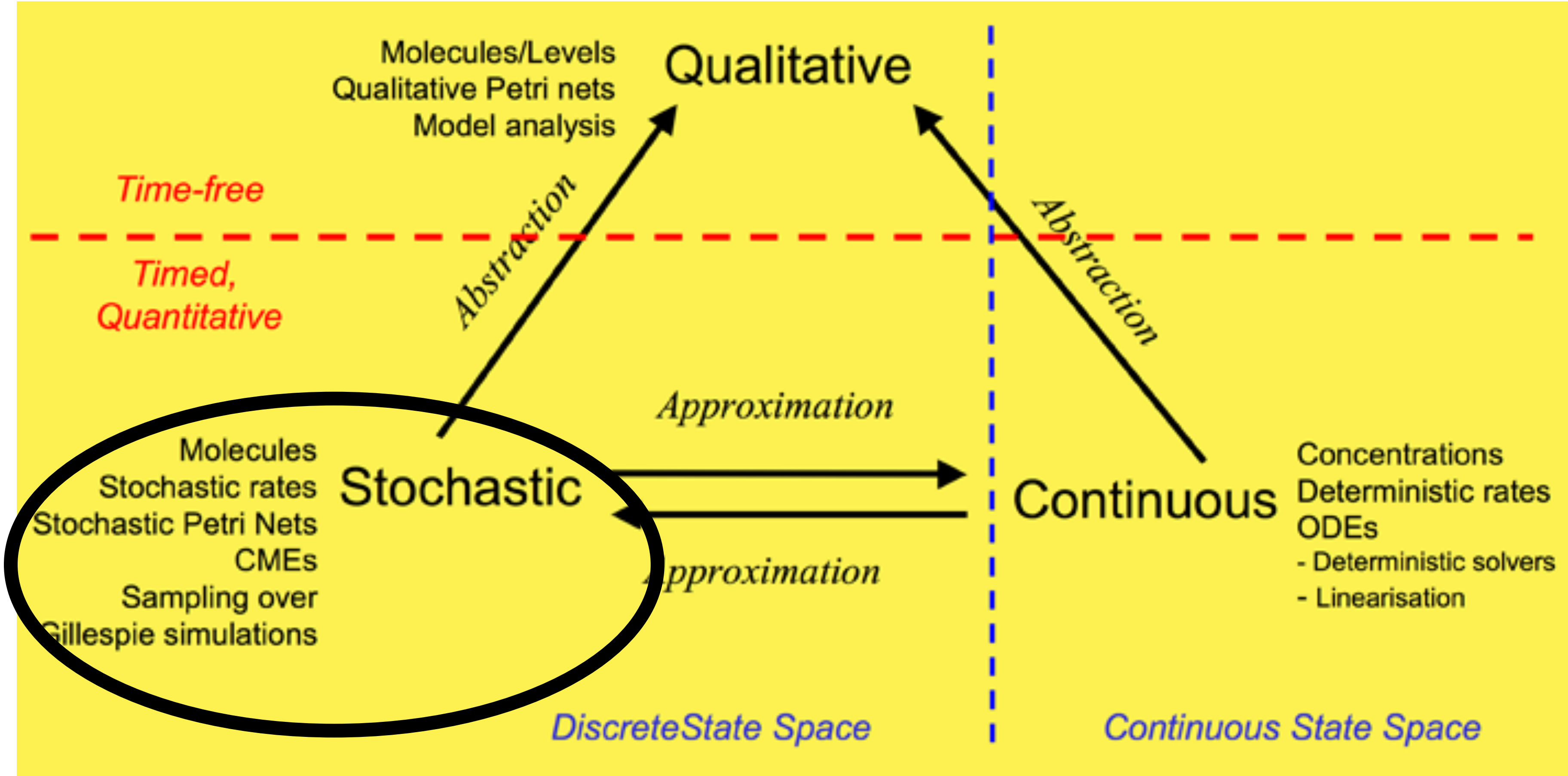


Advantages and disadvantages of stochastic modelling

- 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

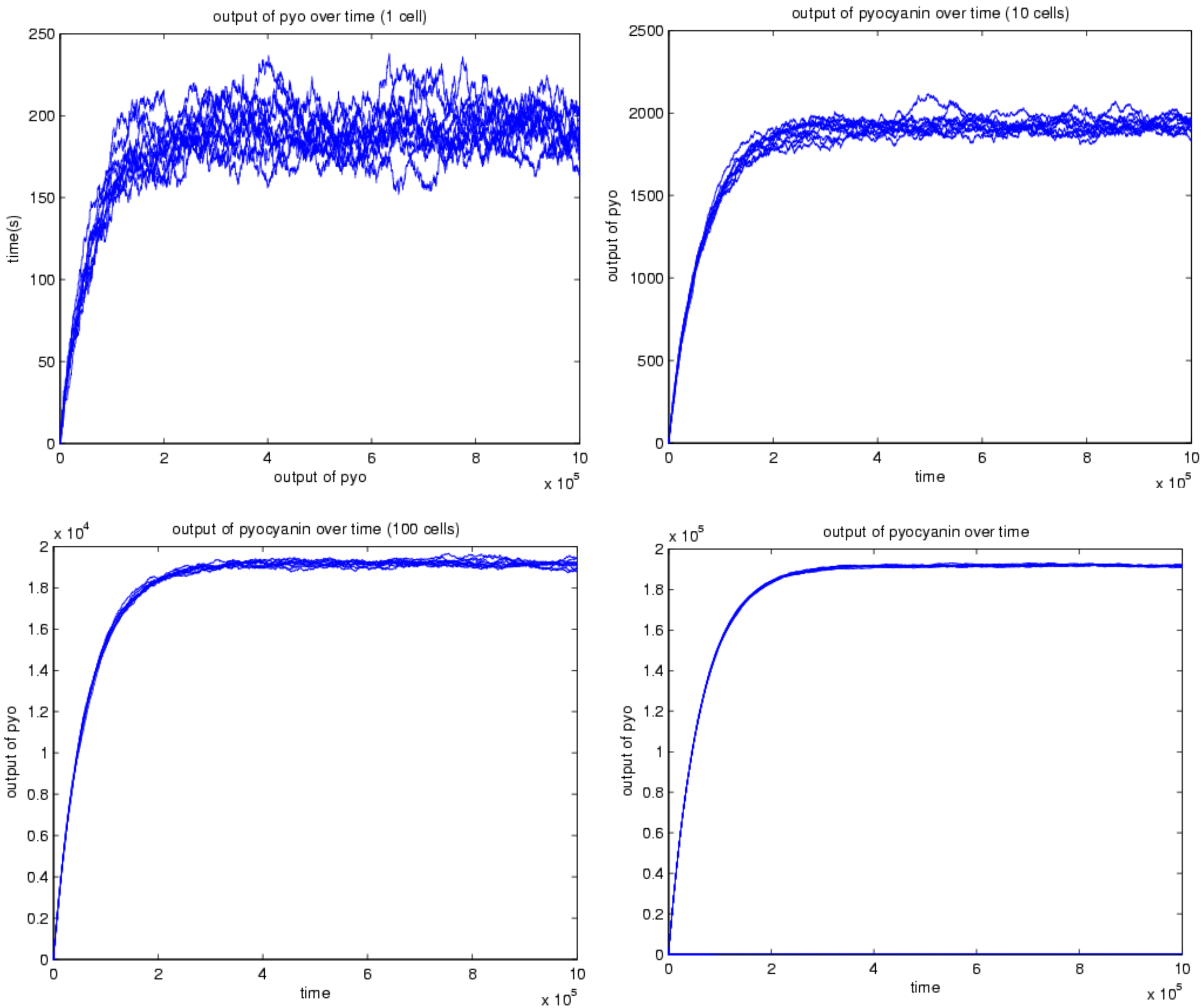
Chemical Master Equations

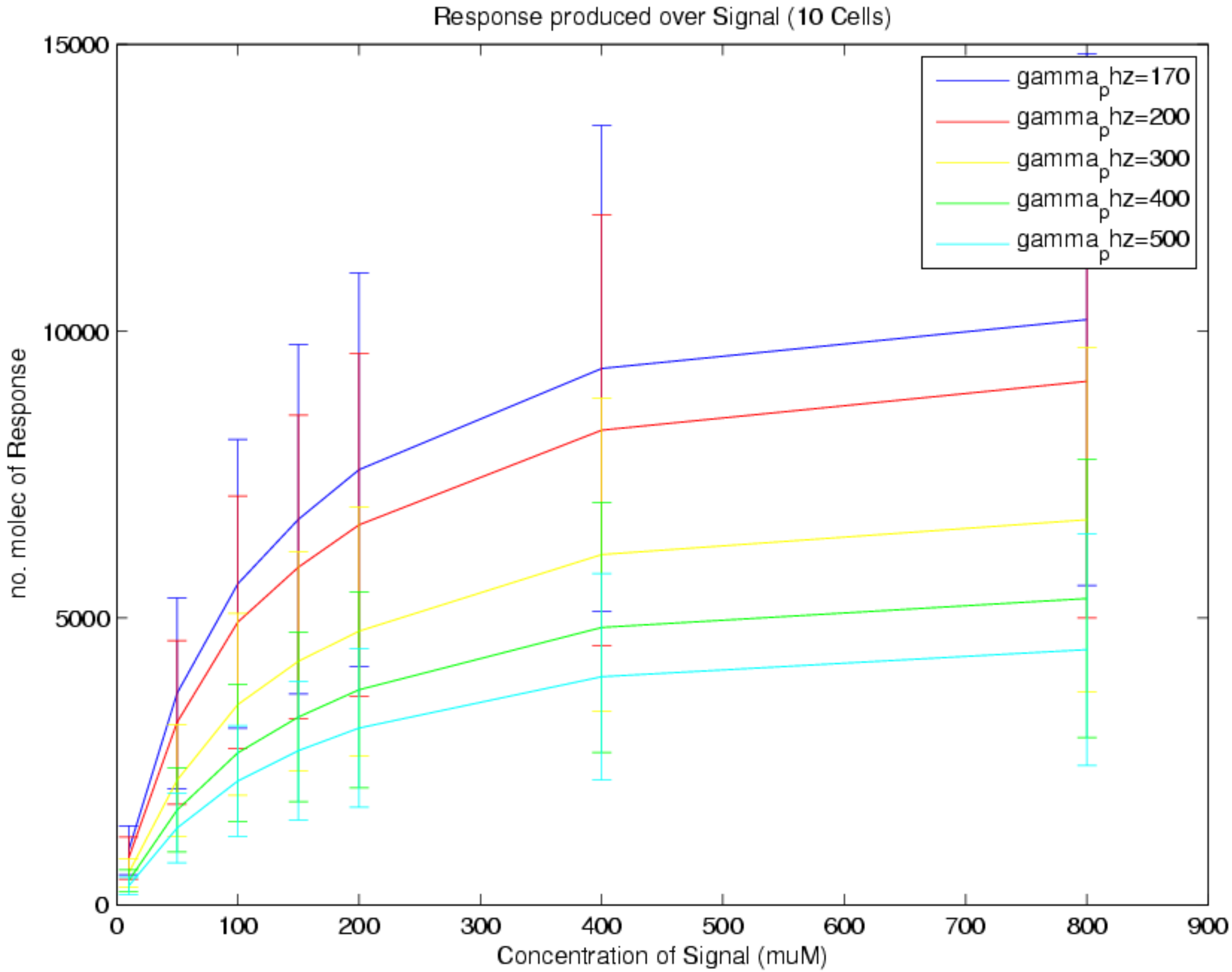
A set of linear, autonomous ODE’s, one ODE for each possible state of the system. The system may be written:

- **Φ → TF** - production of TF
- **TF → Φ** - degradation of TF
- **TF+S → TFS** - association of TFS
- **TFS → TF+S** - dissociation of TFS
- **TFS → Φ** - degradation of TFS
- **Φ → PhzMS** - production of PhzMS
- **PhzMS → Φ** - degradation of PhzMS
- **PhzMS → PYO** - production of pyocyanin
- **PYO → Φ** - degradation of pyocyanin

Propensity Functions

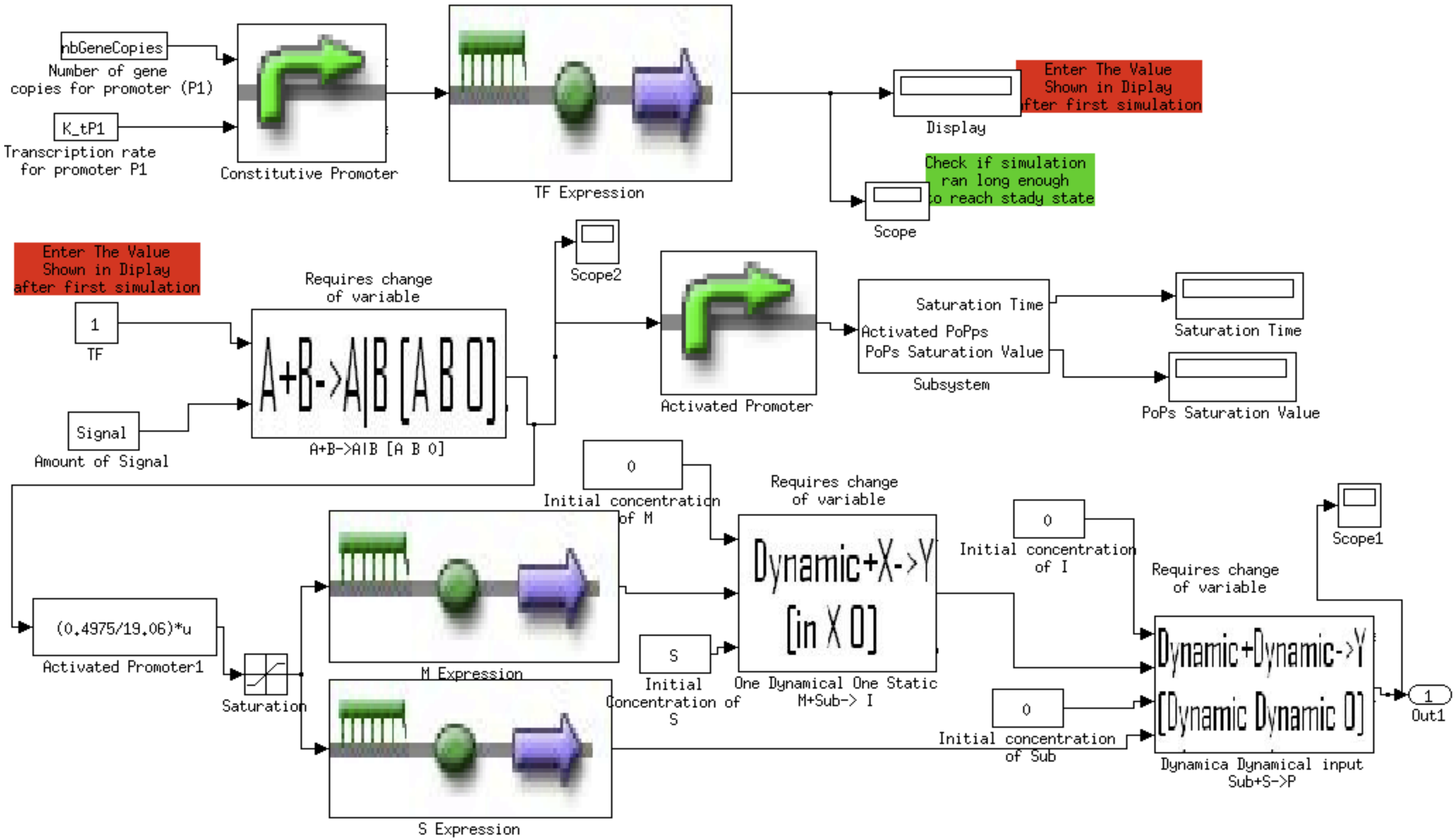
reaction	rate constant	propensity function
$\phi \rightarrow TF$	$\alpha = c(1)$	$a(1) = c(1)$
$TF \rightarrow \phi$	$\delta_{TF} = c(2)$	$a(2) = c(2) * X(1)$
$TF + S \rightarrow TFS$	$K1 * S = c(3)$	$a(3) = c(3) * X(1)$
$TFS \rightarrow TF + S$	$Km1 = c(4)$	$a(4) = c(4) * X(2)$
$TFS \rightarrow \phi$	$\delta_{TFS} = c(5)$	$a(5) = c(5) * X(2)$
$\phi \rightarrow P3$	$\frac{\beta * TFS}{\gamma + TFS} = c(6)$	$a(6) = c(6)$
$P3 \rightarrow \phi$	$\delta_{P3} = c(7)$	$a(7) = c(7) * X(3)$
$P3 \rightarrow P4$	$\alpha_2 = c(8)$	$a(8) = c(8) * X(3)$
$P4 \rightarrow \phi$	$\delta_{P4} = c(9)$	$a(9) = c(9) * X(4)$


output of pyo over time (1 cell)
time(s)
output of pyo
x 10^5
output of pyocyanin over time (10 cells)
output of pyo
time
x 10^5
output of pyocyanin over time (100 cells)
x 10^4
output of pyo
time
x 10^5
output of pyocyanin over time
x 10^5
output of pyo
time
x 10^5



Response produced over Signal (10 Cells)
no. molec of Response
Concentration of Signal (muM)
gamma_phz=170
gamma_phz=200
gamma_phz=300
gamma_phz=400
gamma_phz=500
0
5000
10000
15000
0
100
200
300
400
500
600
700
800
900

Simulink Modelling Environment



In the end…

Our Contributions:

- standard SBML models of the systems
- new biobricks with mathematical description
- Practical comparison of modelling apporaches – 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

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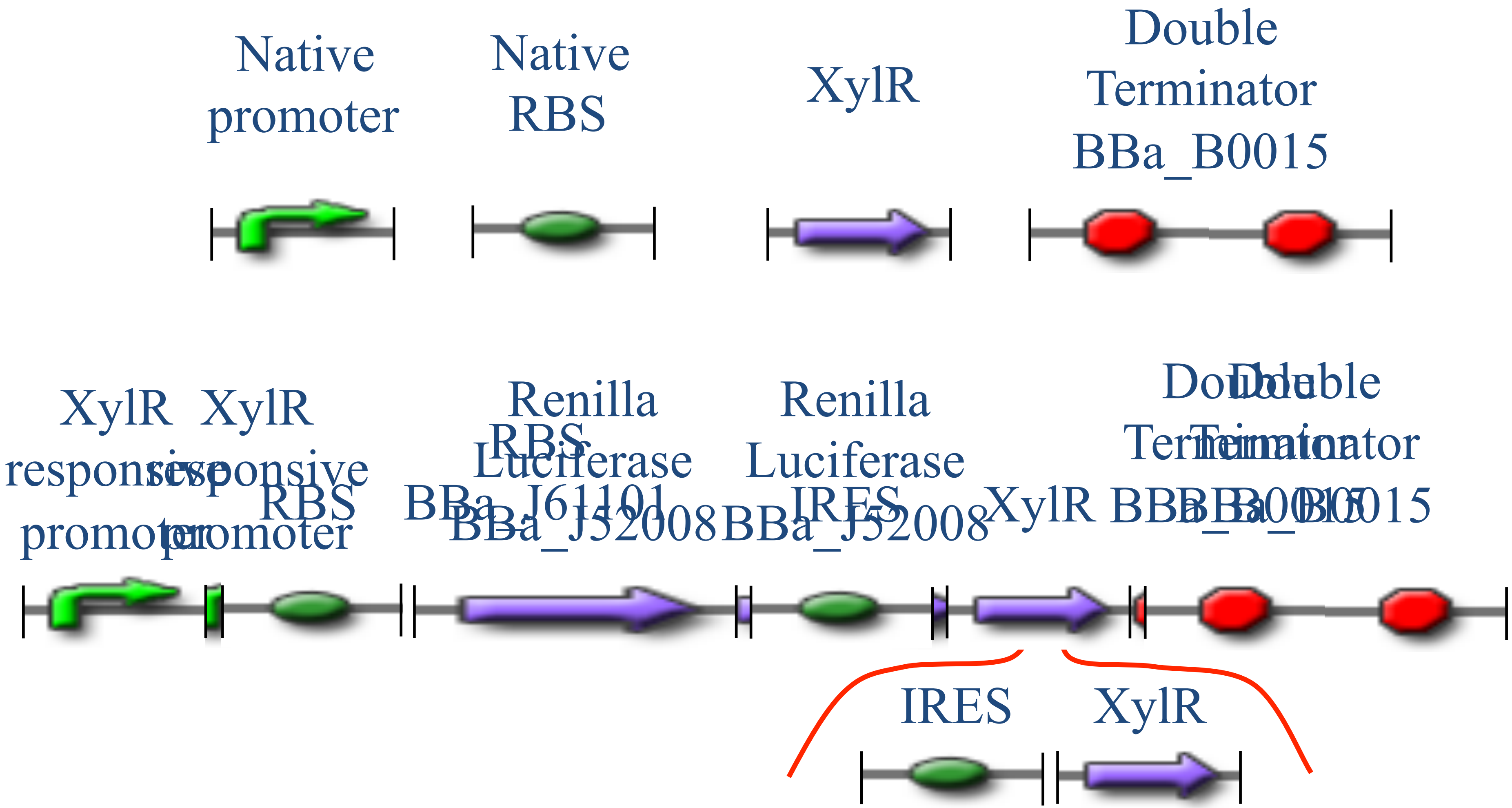
Our Constructs So Far…



Registry Contributions

Number	BioBrick Number	Description
1	BBa_I723032	Xylene-sensitive promoter
2	BBa_I723029	Xylene-sensitive promoter plus RBS
3	BBa_I723023	Xylene-inducible luciferase
4	BBa_I723031	Inducible luciferase
5	BBa_I723024	PhzM
6	BBa_I723025	PhzS
7	BBa_I723026	PhzM plus terminator
8	BBa_I723027	PhzS plus terminator
9	Bba_I723030	Salicylate-inducible transcription factor
10	BBa_I723020	Salicylate-sensitive promoter

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

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- David Gilbert
- Gary Gray
- Xu Gu
- Raya Khanin
- David Leader
- Susan Rosser
- Emma Travis
- Gabriela Kalna