Modelling the dynamic behaviour of biochemical pathways: From analysis to design and construction

David Gilbert
drg@dcs.gla.ac.uk

Bioinformatics Research Centre University of Glasgow

- Systems Biology will play a major role in providing a framework for engineering biological systems

  - To what extent can Systems Biology be regarded as a rigorous discipline incorporating sound analytical principles?
  - Explore contributions to Systems Biology.

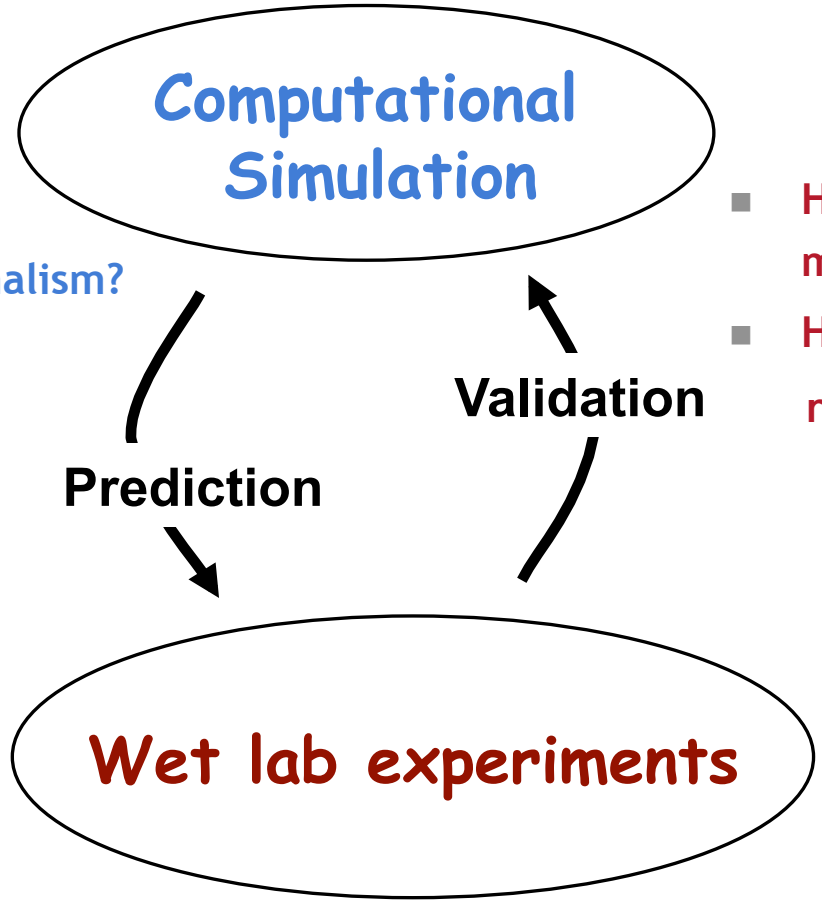
- Illustrations
  - MAPK signal transduction pathway
  - Synthetic biology: self-powered pollution sensor

Modelling the kinetic behaviour of the MAPK cascade: Negative Feedback Amplifer

- Hypothesis: feedback amplifier
- “Similarity” to electronic circuit theory
- Continuous cross check between modelling and real experimental data obtained from in-vivo experiments
- Prediction: perturbation by pharmacological intervention should be less effective within the feedback - amplifier module than outside of it.

Biochemical Pathway Simulation

- What is the best formalism?
- How to deal with lack of information?
- Predictions on what?



- How to collect quantitative measurements in vivo?
- How to manipulate regulatory mechanisms?

Dynamic behaviour - Modelling

- Ordinary Differential Equations
- Stochastic process algebras
- Petri nets

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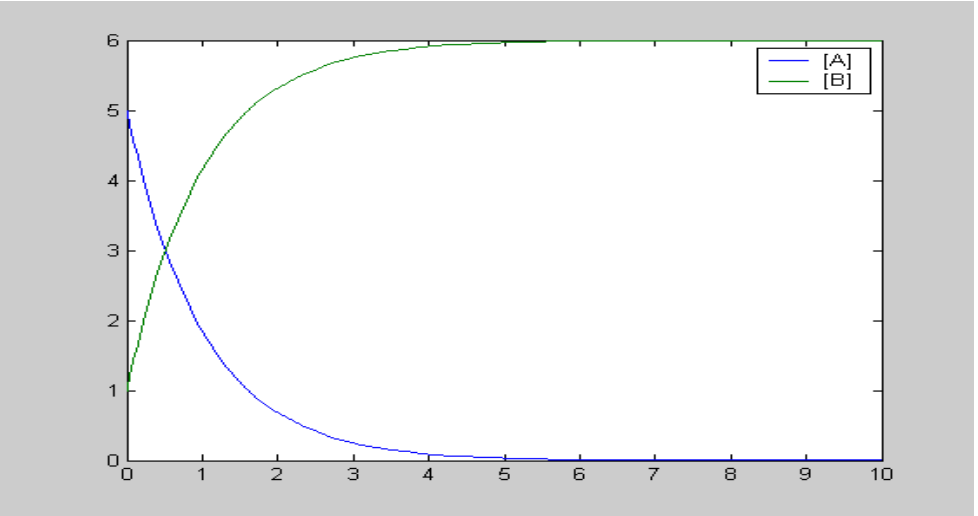
Kinetics and differential equations

Concentration of Molecule A = [A], usually in units mol/litre (molar)

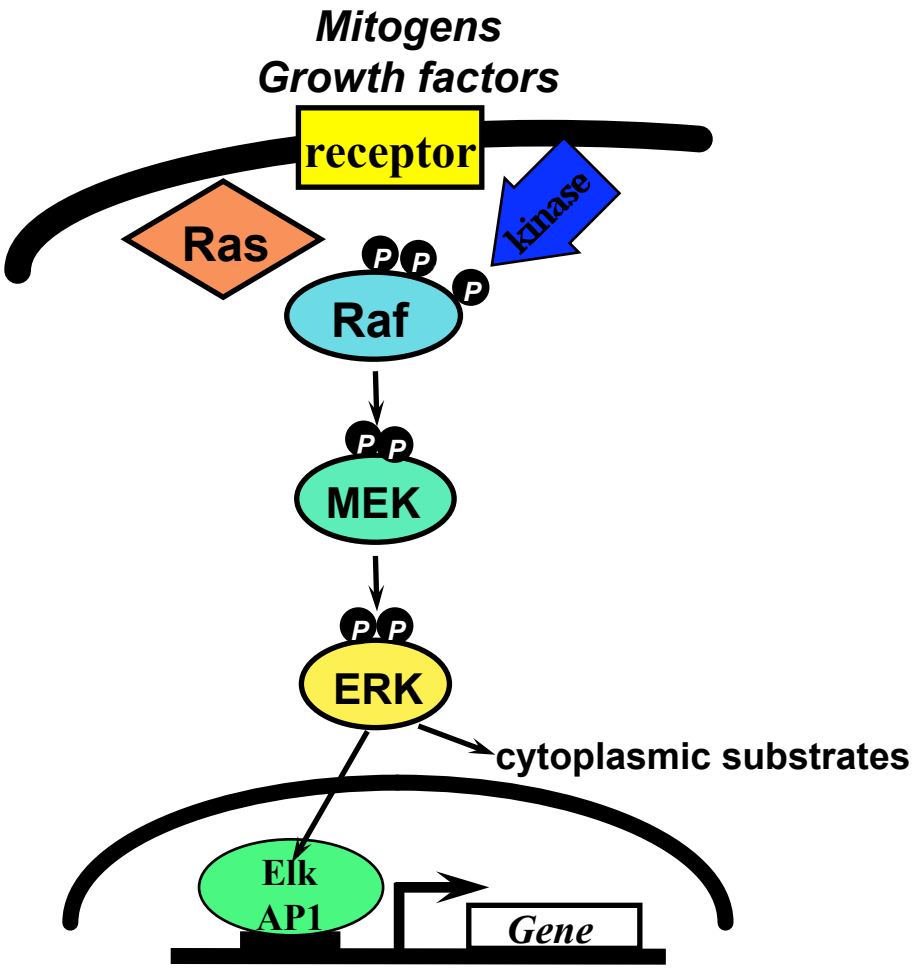
Rate constant = k, with indices indicating constants for various reactions (k_1, k_2...)

$$A \rightarrow B$$

$$\frac{d[A]}{dt} = -\frac{d[B]}{dt} = -k_1[A]$$

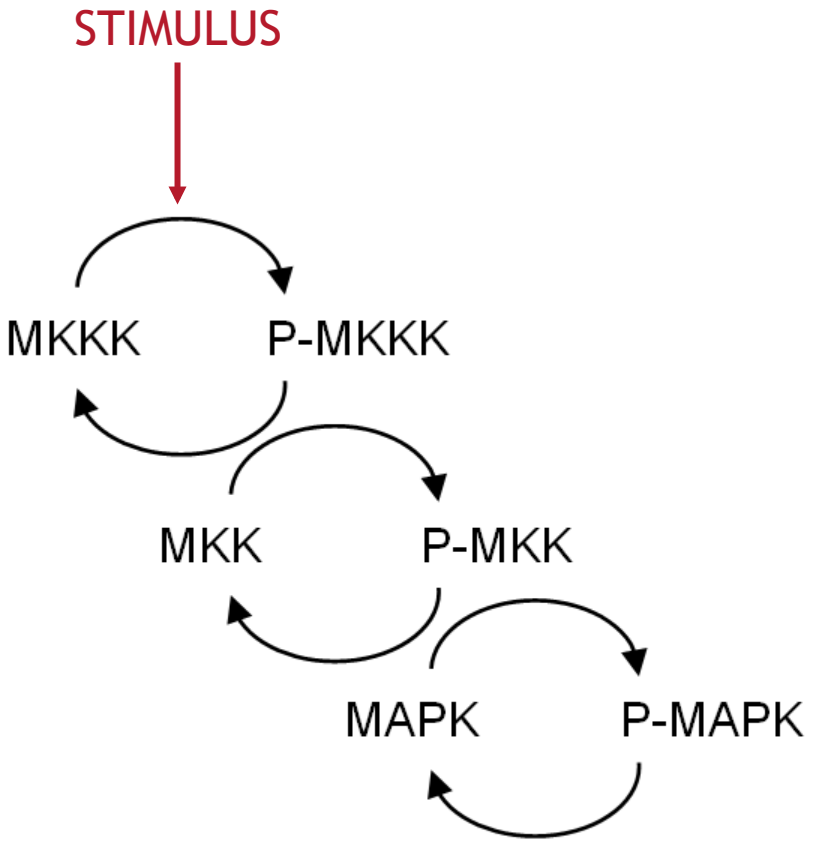


The Ras – Raf – MEK – ERK Signalling pathway



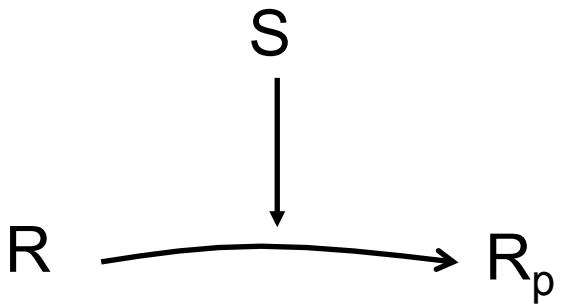
MAPK Pathway

- Responds to wide range of stimuli: cytokines, growth factors, neurotransmitters, cellular stress and cell adherence,…
- Pivotal role in many key cellular processes:
  - growth control in all its variations,
  - cell differentiation and survival
  - cellular adaptation to chemical and physical stress.
- Deregulated in various diseases: cancer; immunological, inflammatory and degenerative syndromes,
- Represents an important drug target.



Mass action for enzymatic reaction - phosphorylation

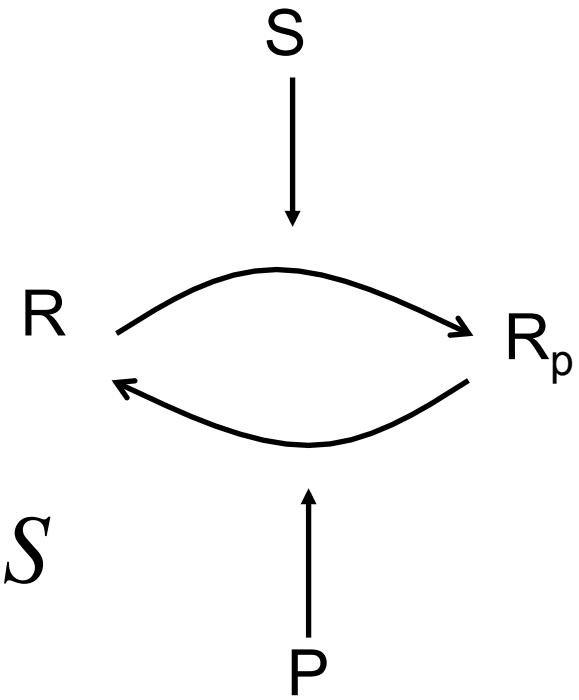
- R: substrate,
- R_p: product (phosphorylated R)
- S: enzyme (kinase)
- R|S substrate-enzyme complex



$$R + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} R \mid S \xrightarrow{k_3} R_p + S$$

Phosphorylation - dephosphorylation step
Mass action model 1

- R: unphosphorylated form
- R_p: phosphorylated form
- S: kinase
- P: phosphotase
- R|S unphosphorylated+kinase complex
- R|P unphosphorylated+phosphotase complex



$$R+S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R|S \xrightarrow{k_3} R_p + S$$

$$R+P \xleftarrow{k_3'} R_p|P \underset{k_2'}{\overset{k_1'}{\rightleftharpoons}} R_p + P$$

Phosphorylation - dephosphorylation step
Mass action model 1

```
dydt = [-k1*S*R + k2*RS + k3*RS                          % S
        -k1*S*R + k2*RS + k3dash*RpP                     % R
        +k1*S*R - k2*RS - k3*RS                          % RS
        -k1dash*P*Rp + k2dash*RpP + k3*RS                % Rp
        -k1dash*P*Rp + k2dash*RpP + k3dash*RpP           % P
        +k1*Rp*P - k2dash*RpP - k3dash*RpP ];            % RpP
```

$$R + S \xrightarrow{k_1} R \mid S \qquad R_p + P \xrightarrow{k_1'} R_p \mid P$$

$$R \mid S \xrightarrow{k_2} R + S \qquad R_p \mid P \xrightarrow{k_2'} R_p + P$$

$$R \mid S \xrightarrow{k_3} R_p + S \qquad R_p \mid P \xrightarrow{k_3'} R + P$$

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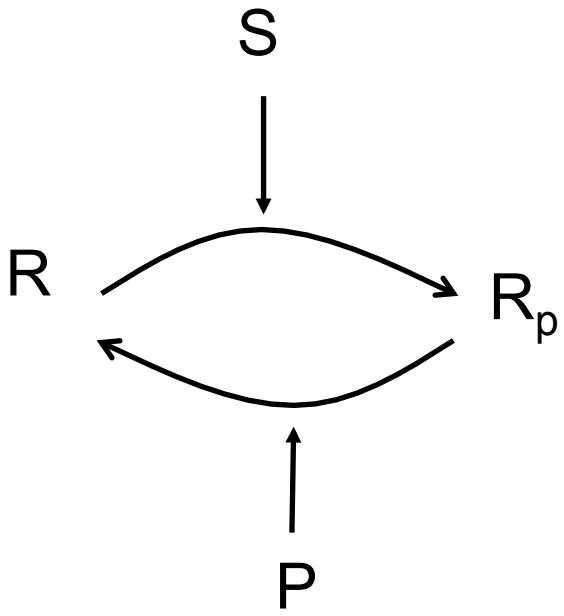
Phosphorylation - dephosphorylation step
Mass action (all singing/dancing)

- R: unphosphorylated form
- R_p: phosphorylated form
- S: kinase
- P: phosphotase
- R|S unphosphorylated+kinase complex
- R|P unphosphorylated+phosphotase complex

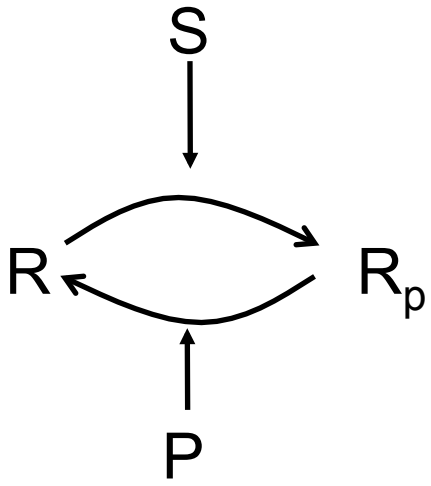


$$R+S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R\,|\,S \underset{k_6}{\overset{k_3}{\rightleftharpoons}} R_p\,|\,S \underset{k\,5}{\overset{k_4}{\rightleftharpoons}} R_p + S$$

$$R+P \underset{k_5'}{\overset{k_4'}{\rightleftharpoons}} R\,|\,P \underset{k_6'}{\overset{k_3'}{\rightleftharpoons}} R_p\,|\,P \underset{k_2'}{\overset{k_1'}{\rightleftharpoons}} R_p + P$$

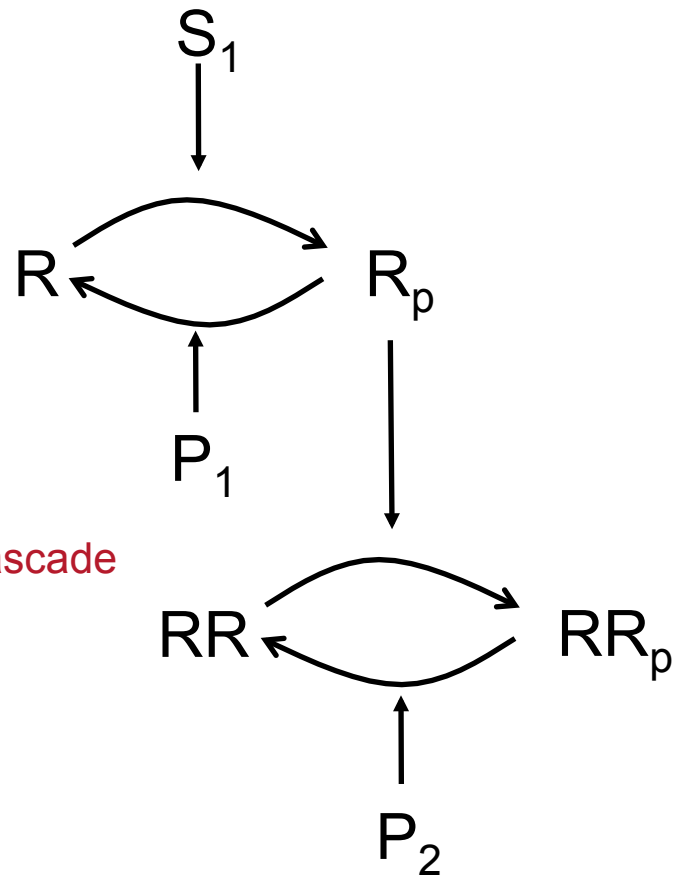
Michaelis-Menten equation for phosphorylation-dephosphorylation

$$\frac{dR_p}{dt} = \frac{k_3 \times S \times R}{K_{m1} + R} - \frac{k_3' \times R_p}{K_{m2} + R_p}$$

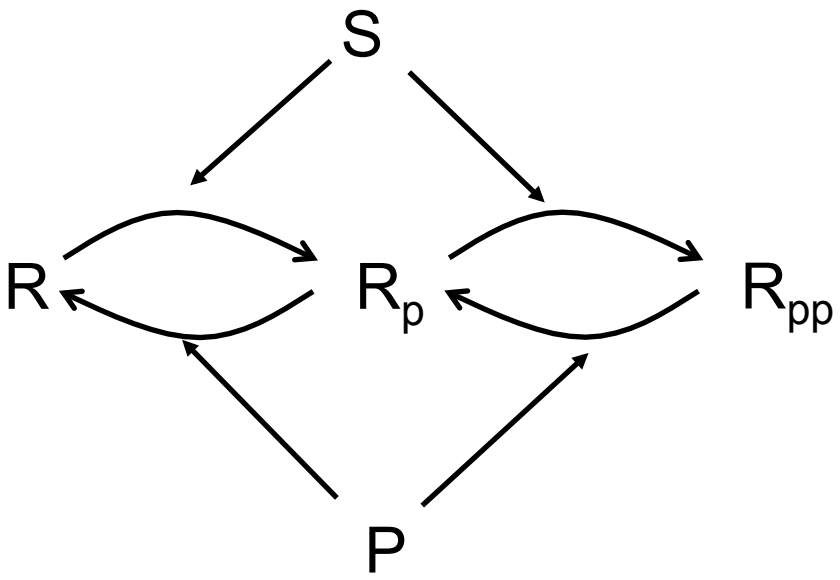


- Assumptions:
  1. No product reverts to initial substrate
  2. MM Equation holds at initial stage of reaction before concentration of product is appreciable
  3. [Enzyme] << [Substrate]
- K_m is [Substrate] at which the reaction rate is half its maximum value
- dR_p/dt == reaction rate V
- k_3 x S == V_{max} for the forward reaction
- k_3' == V_{max} for the reverse reaction (Phosphotase is ignored)
- K_{m1} == $(k_2+k_3)/k_1$ (k's from mass-action 1)

Composition
vertical & horizontal



2-stage cascade



1-stage cascade
double phosphorylation step

3-stage Phosphorylation cascade (Mass Action)

$$R + S_1 \underset{k_2}{\overset{k_1}{\rightleftarrows}} R \mid S_1 \xrightarrow{k_3} R_p + S_1$$

$$R + P_1 \xleftarrow{k_3'} R_p \mid P_1 \underset{k_2'}{\overset{k_1'}{\rightleftarrows}} R_p + P_1$$

$$S_2 = R_p$$

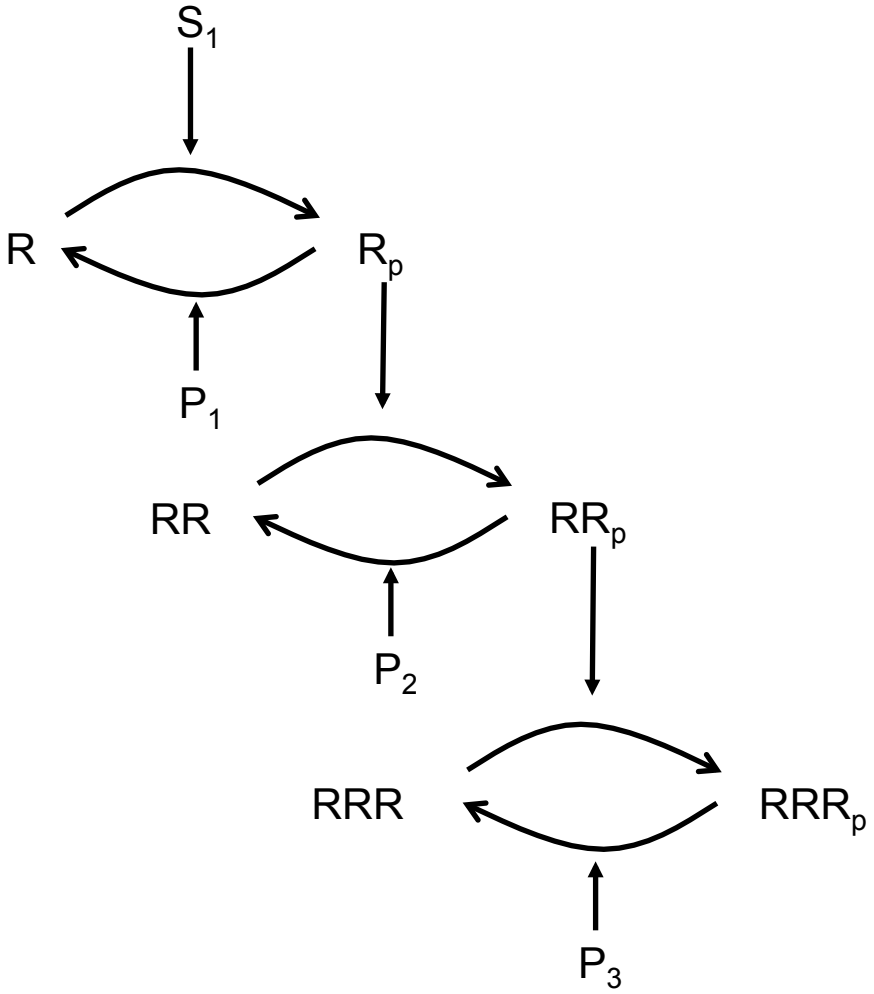
$$RR + S_2 \underset{kk_2}{\overset{kk_1}{\rightleftarrows}} RR \mid S_2 \xrightarrow{kk_3} RR_p + S_2$$

$$RR + P_2 \xleftarrow{kk_3'} RR_p \mid P_2 \underset{kk_2'}{\overset{kk_1'}{\rightleftarrows}} RR_p + P_2$$

$$S_3 = RR_p$$

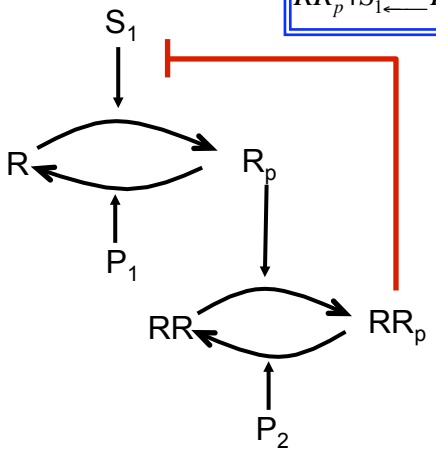
$$RRR + S_3 \underset{kkk_2}{\overset{kkk_1}{\rightleftarrows}} RRR \mid S_3 \xrightarrow{kkk_3} RRR_p + S_3$$

$$RRR + P_3 \xleftarrow{kkk_3'} RRR_p \mid P_3 \underset{kkk_2'}{\overset{kkk_1'}{\rightleftarrows}} RRR_p + P_3$$



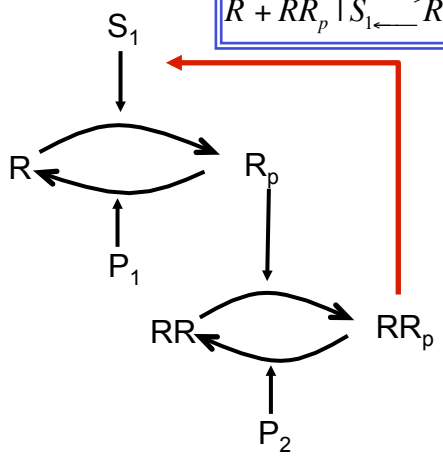
Phosphorylation cascade + feedback

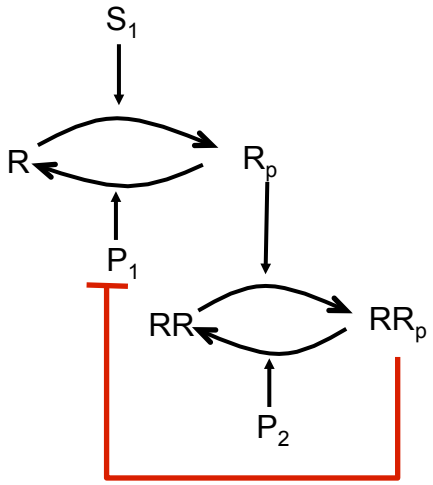
$$RR_p + S_1 \rightleftarrows RR_p \mid S_1$$



$$RR_p + S_1 \rightleftarrows RR_p \mid S_1$$

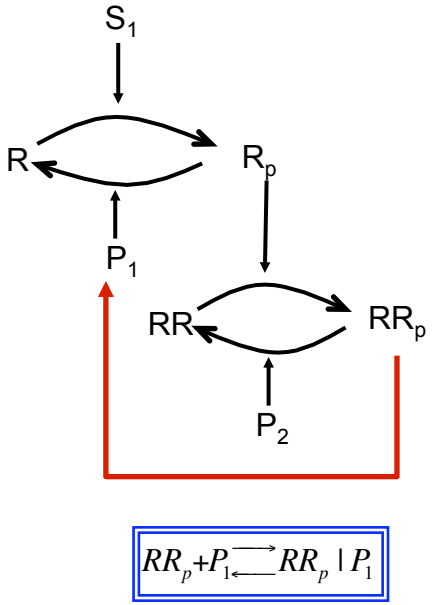
$$R + RR_p \mid S_1 \rightleftarrows R \mid RR_p \mid S_1 \longrightarrow RR_p \mid S_1$$





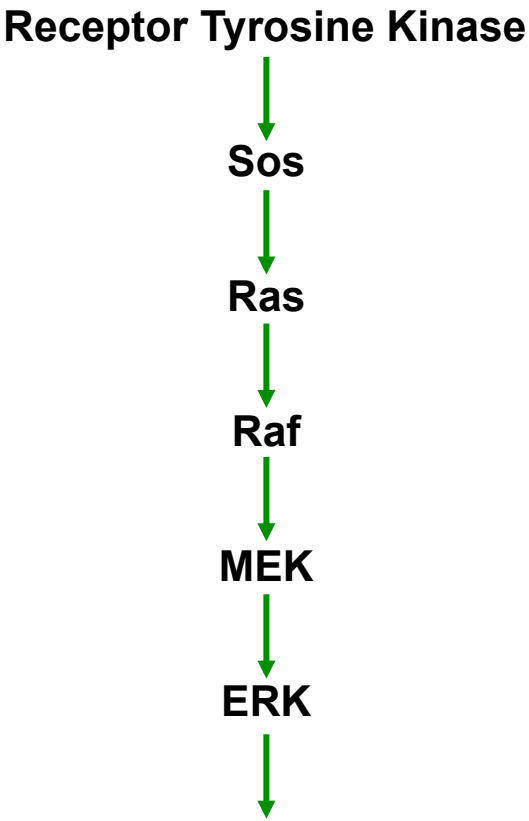
$$RR_p + P_1 \rightleftarrows RR_p \mid P_1$$

$$R_p + RR_p \mid P_1 \rightleftarrows R_p \mid RR_p \mid P_1 \longrightarrow RR_p \mid P_1$$



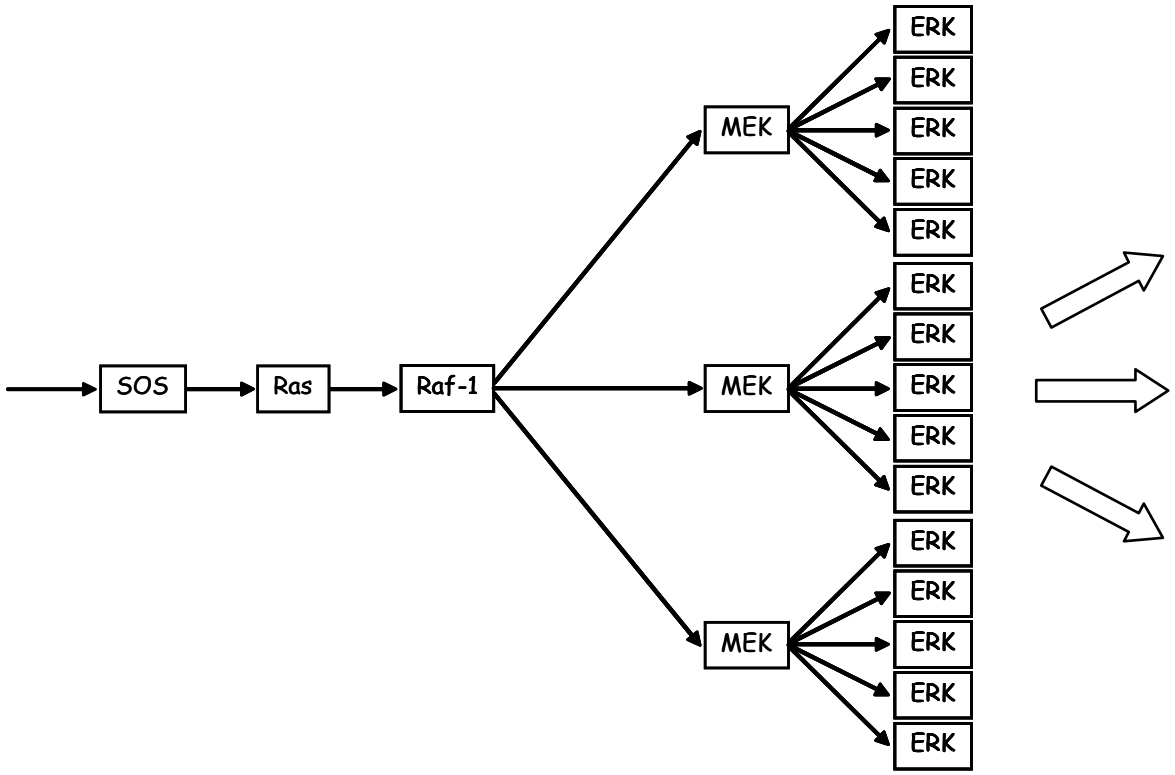
$$RR_p + P_1 \rightleftarrows RR_p \mid P_1$$

ERK Cascade



Amplification

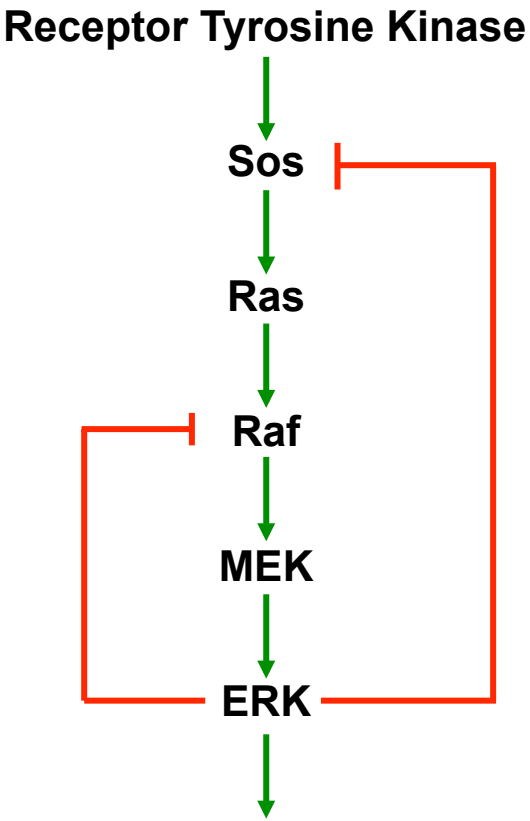
- ERK cascade well known biological amplifier -- amplifies the original signal to create effective cellular responses.
- 1:3:5 are the approximate ratios of Raf-1, MEK and ERK in fibroblasts.



Negative Feedback

- Well known negative feedback loop: phosphorylation of SOS by ERK-PP (via MAPKAP1) resulting in the dissociation of the Grb2/SOS complex.

- New negative feedback loop: ERK-PP phosphorylates Raf-1 resulting in a hyper-phosphorylated inactive form of Raf (Dougherty *et al*. 2005)



Dougherty *et al*. (2005), Regulation of Raf-1 by Direct Feedback Phosphorylation, Molecular Cell 17 215-224

Is the ERK pathway a negative feedback amplifier?

Sauro HM, Kholodenko BN.
Quantitative analysis of signaling networks.
Prog Biophys Mol Biol. 2004 Sep;86(1):5-43.

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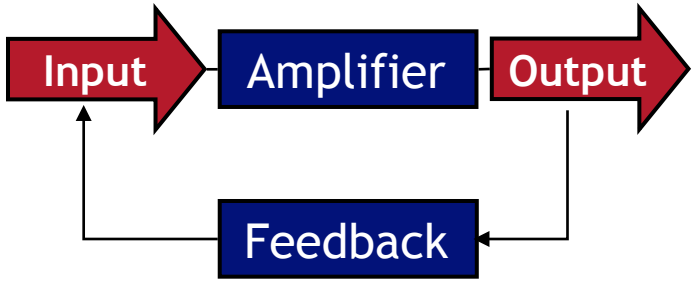
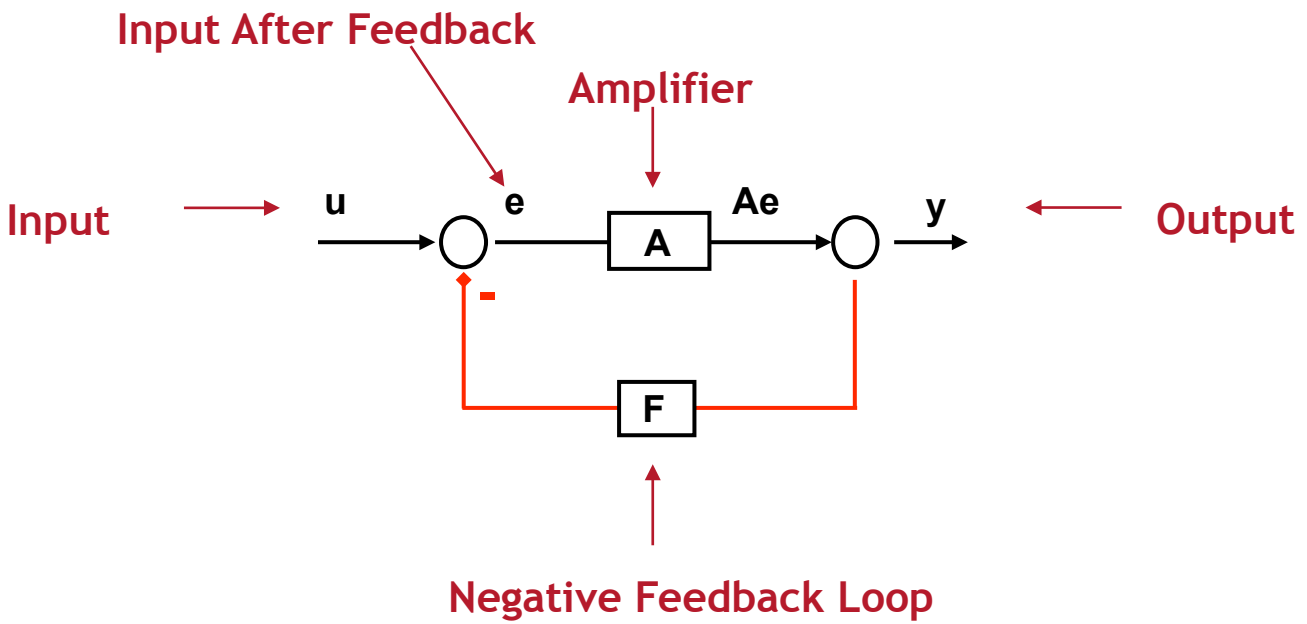
Negative Feedback Amplifier

- A negative feedback amplifier stems from the field of electronics and consists of an amplifier with a negative feedback loop from the output of the amplifier to its input.
- The negative feedback loop results in a system that is much more robust to disturbances in the amplifier.
- The negative feedback amplifier was invented in 1927 by Harold Black of Western Electric and was originally used for reducing distortion in long distance telephone lines.
- The negative feedback amplifier is now a key electrical component used in a wide variety of applications



Negative Feedback Amplifier



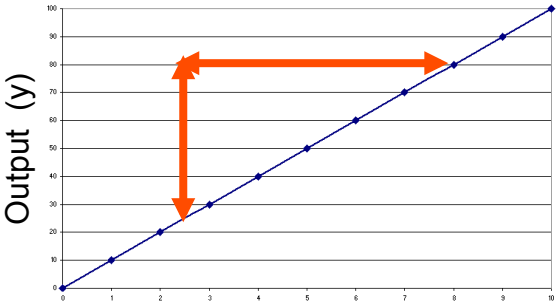
Steady State Equation $$y = \frac{Au}{1 + AF}$$

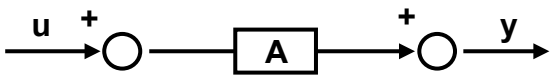
$y = Ae$
$e = u - Fy$

$y = A(u - Fy)$
$y = Au - AFy$
$y + AFy = Au$
$y(1 + AF) = Au$

The negative feedback imparts signalling robustness

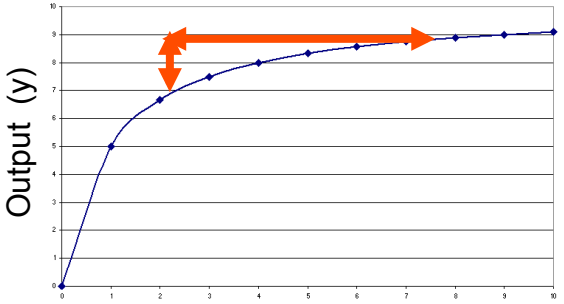
Standard Amplifier

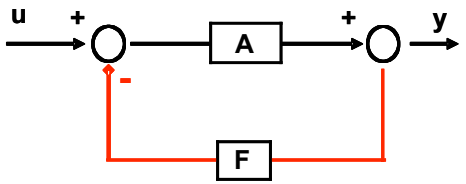




y=A*u

Negative Feedback Amplifier

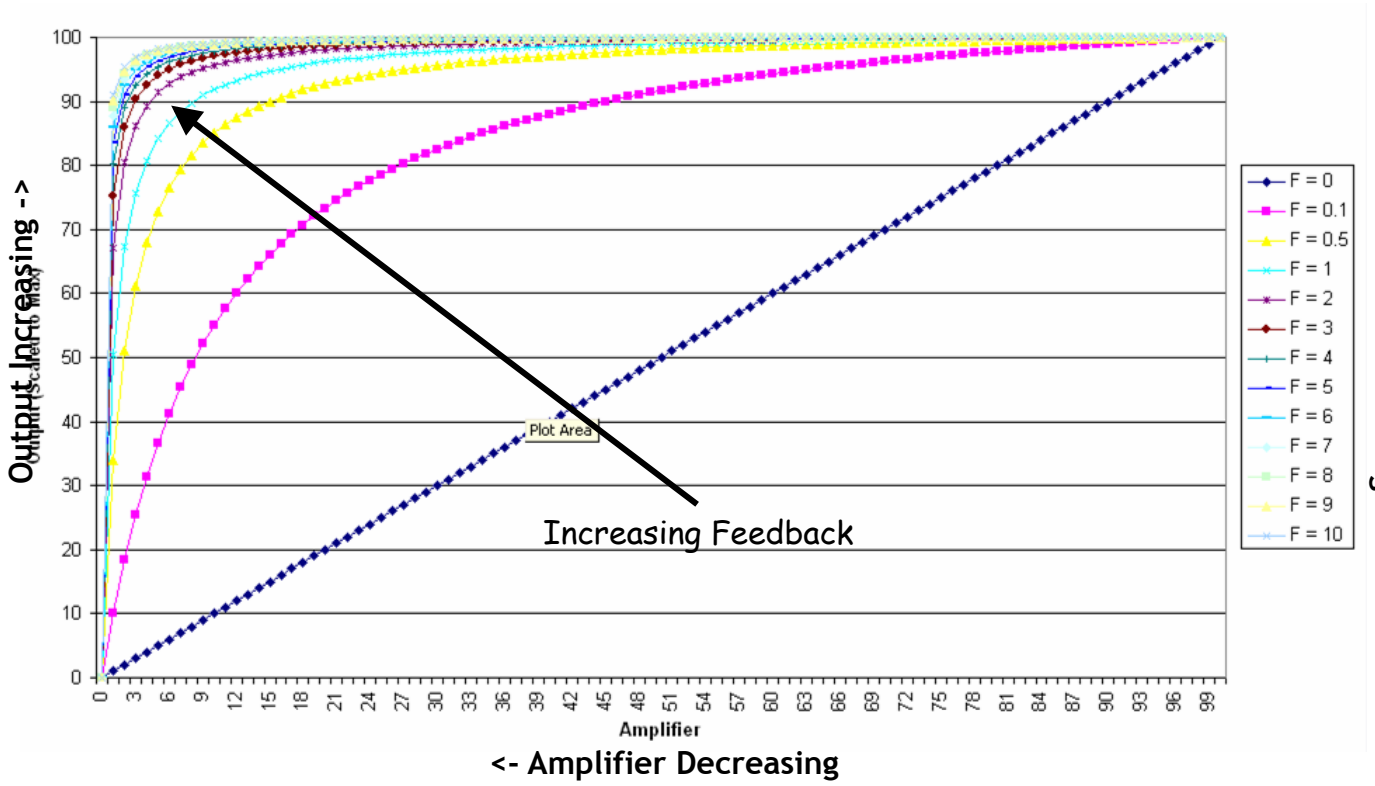




y=A*u/(1+A*F)

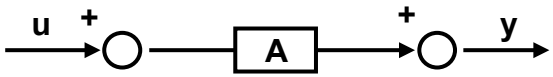
A large change in amplifier gain leads to a small change in output (y)

Feedback


Output Increasing ->
Amplifier
<- Amplifier Decreasing
Feedback Increasing ->
Plot Area
Increasing Feedback
F = 0
F = 0.1
F = 0.5
F = 1
F = 2
F = 3
F = 4
F = 5
F = 6
F = 7
F = 8
F = 9
F = 10

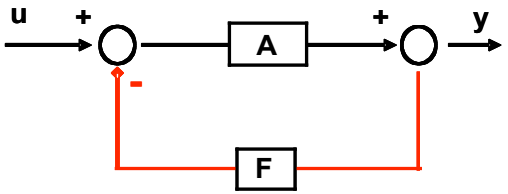
The negative feedback imparts signalling robustness

Standard Amplifier



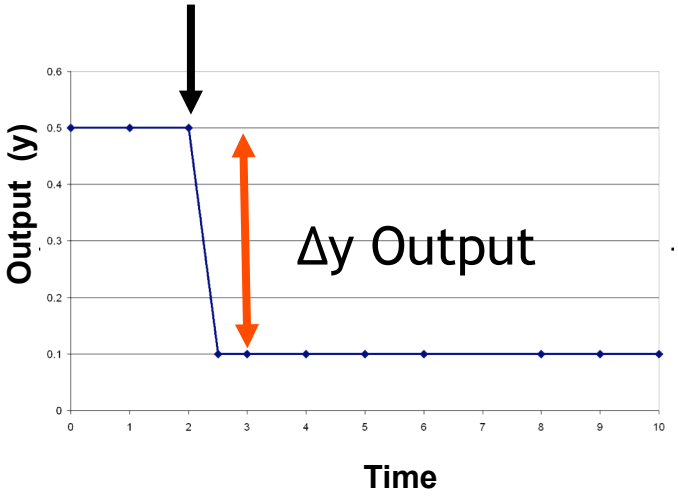
y=A*u

Negative Feedback Amplifier

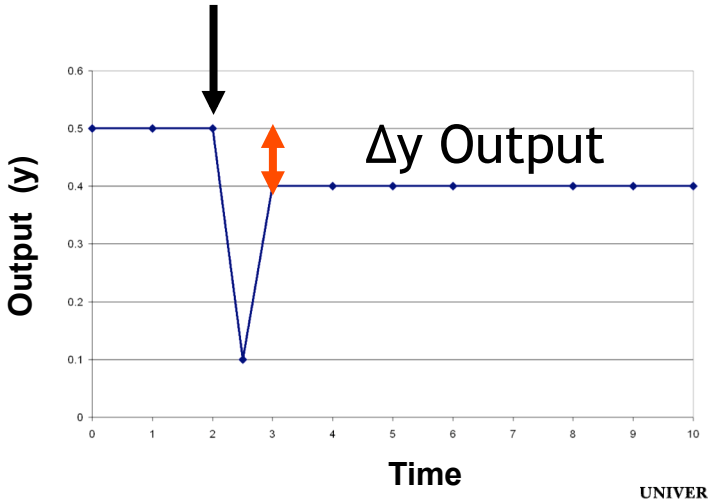


y=A*u/(1+A*F)

Sudden drop in Amplifier (A) gain



Sudden drop in Amplifier (A) gain



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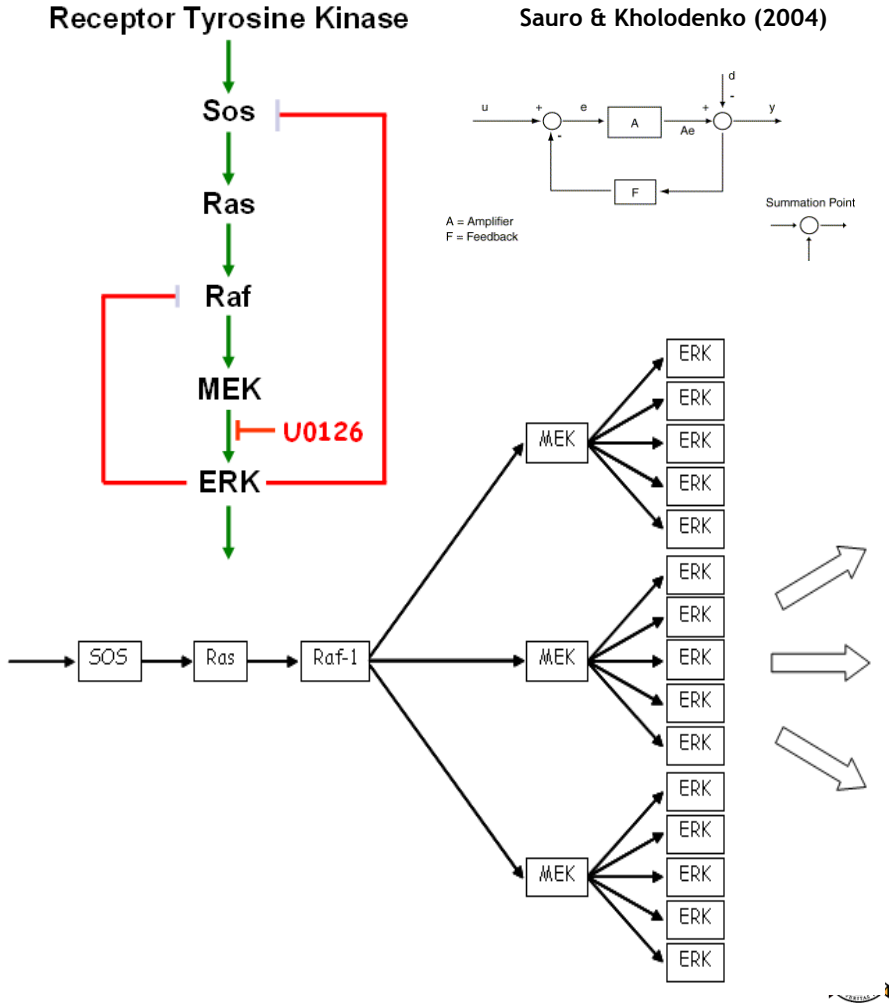
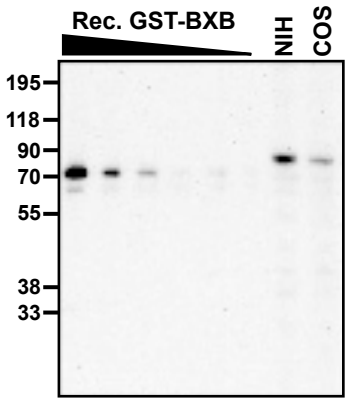
Application to Biology

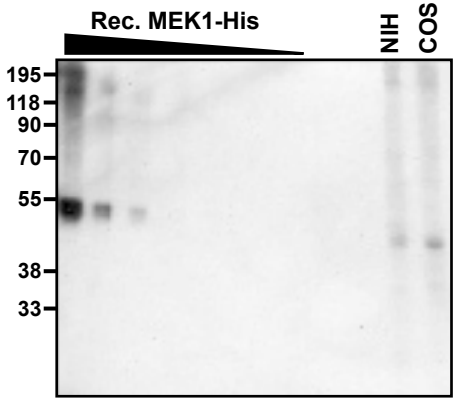
- The ERK cascade is a well known biological amplifier and contains numerous negative feedback loops.
- At first sight, it has the correct structure to be a negative feedback amplifier.
- If the ERK cascade is a negative feedback amplifier it should be robust to disturbances within the cascade.
- From a biological point of view, these disturbances could be caused by drugs, such as U0126, aimed at decreasing the activity of the ERK cascade.
- This suggests that these drugs will be relatively ineffective.
- In fact, current drugs aimed at decreasing the activity of the MAPK pathway have proved less efficient in *in vivo* applications than anticipated from *in vitro* inhibition assays.



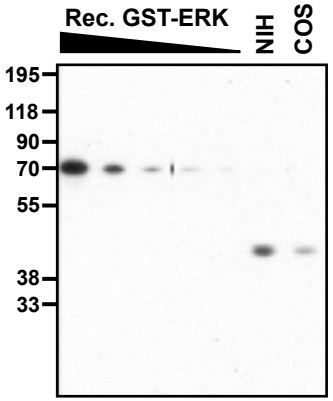
Raf/MEK/ERK amplifies the signal



WB: Raf-1



WB: MEK

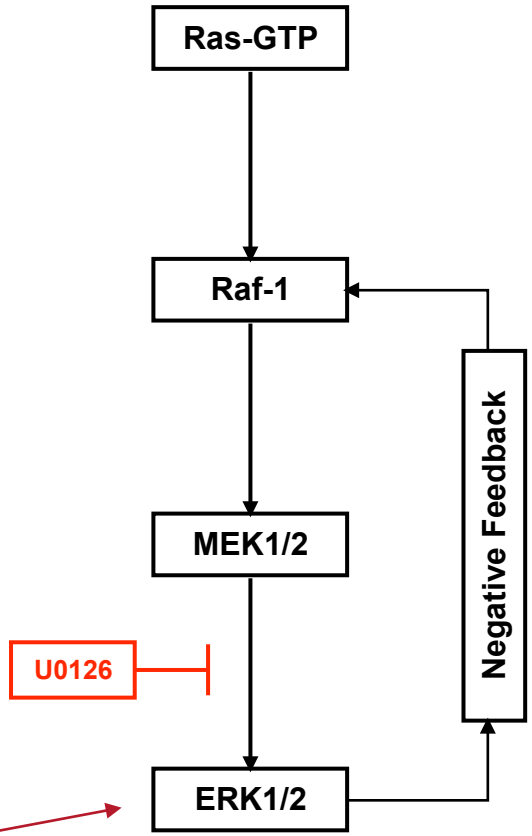


WB: ERK

Cell line	Raf-1	MEK	ERK	Concentration per cell
COS1	3.6	10.6	21.2	femtomol
	1	2.9	5.9	ratio
NIH 3T3	10.9	7.1	98	femtomol
	1	0.7	9	ratio

How to test if the ERK pathway is a NFA?

Generate input:
Stimulate with GF



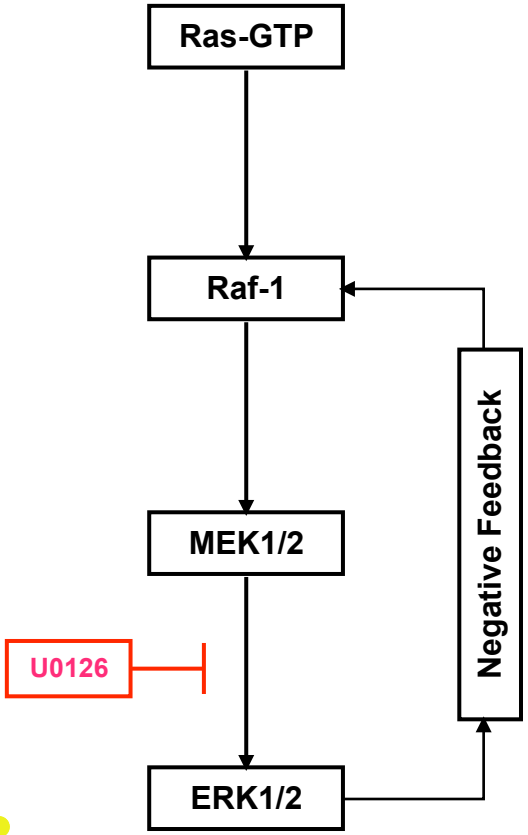
Remove negative feedback

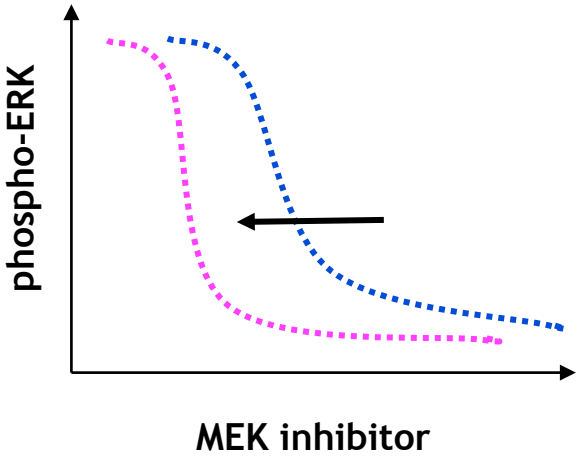
"Disturb the Amplifier":
Use a MEK inhibitior, such as U0126

Measure signal output:
i.e. ERK phosphorylation

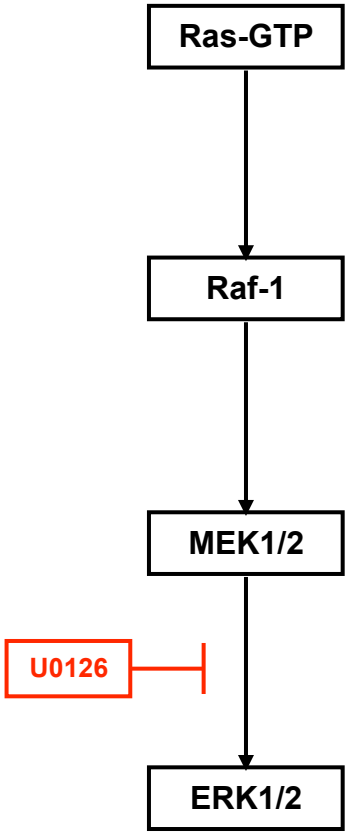
Hypothesis: Braking the feedback should sensitise the ERK pathway to MEK-inhibitor

Feedback intact





Feedback removed



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How to test if the ERK pathway is a NFA?

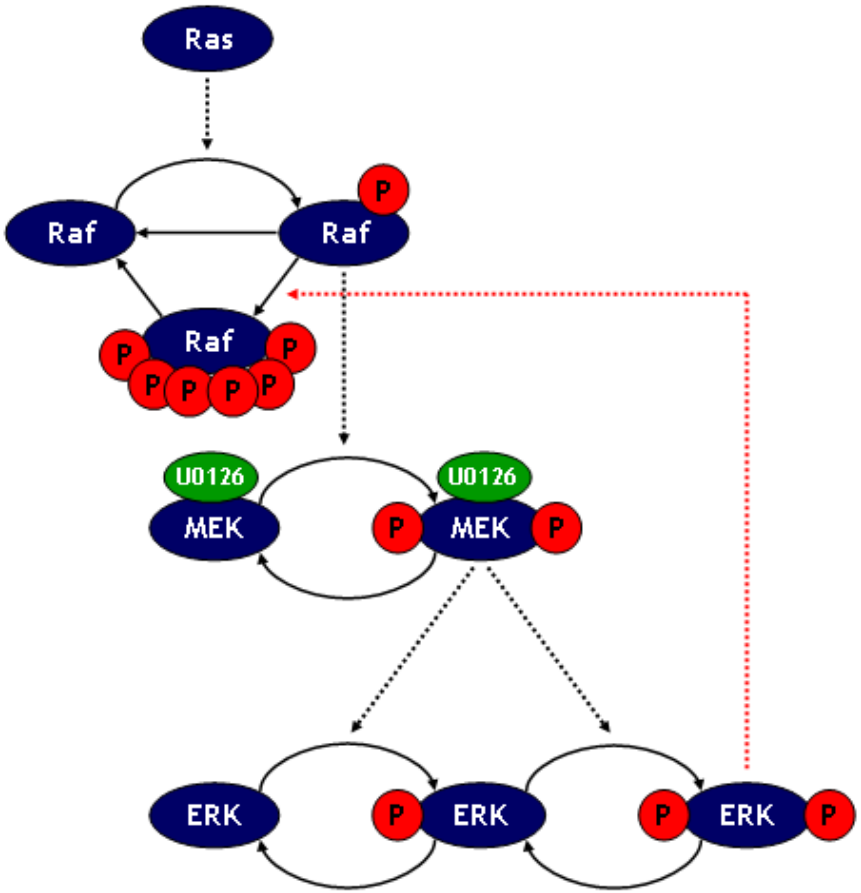
Strategy

In vivo system that allows us to compare feedback broken to feedback intact model.

Computational Model of ERK pathway with/without feedback

Computational Modeling 1: Build the model

- Non-linear ordinary differential equations (ODE's).
- ODE's were solved using Math Lab and Gepasi.
- Models are based on the Schoeberl et al. (2002) model
- Mass Action Kinetics instead of Michaelis Menten
- Kinetic parameters are from literature, previous models and "guesstimates"



Schoeberl *et al*. (2002), Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors, Nature Biotechnology 20, 370-375

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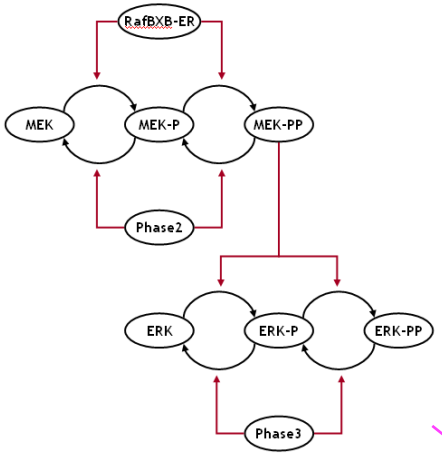
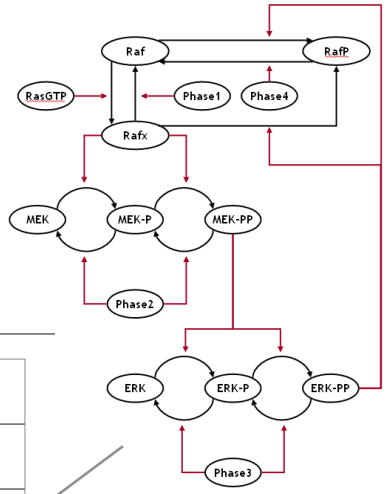
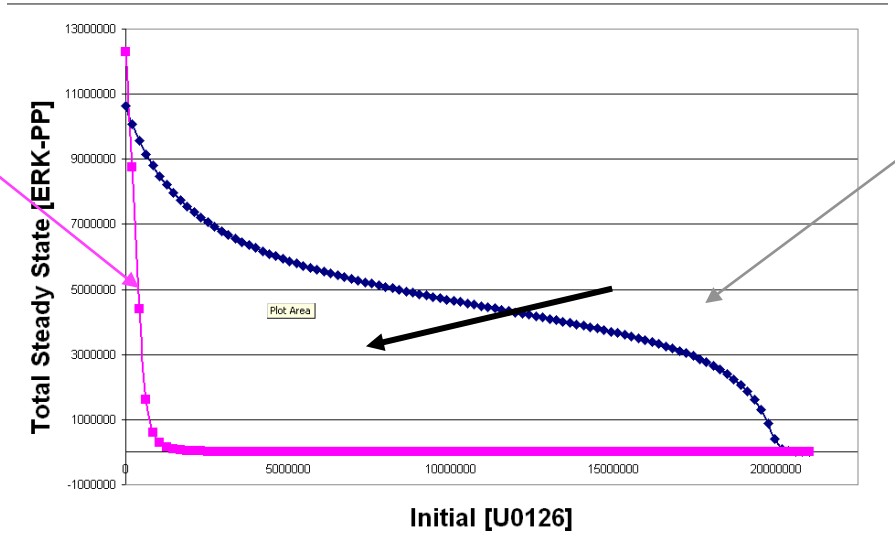
Computational Modeling 2: Results

Feedback broken



Feedback intact

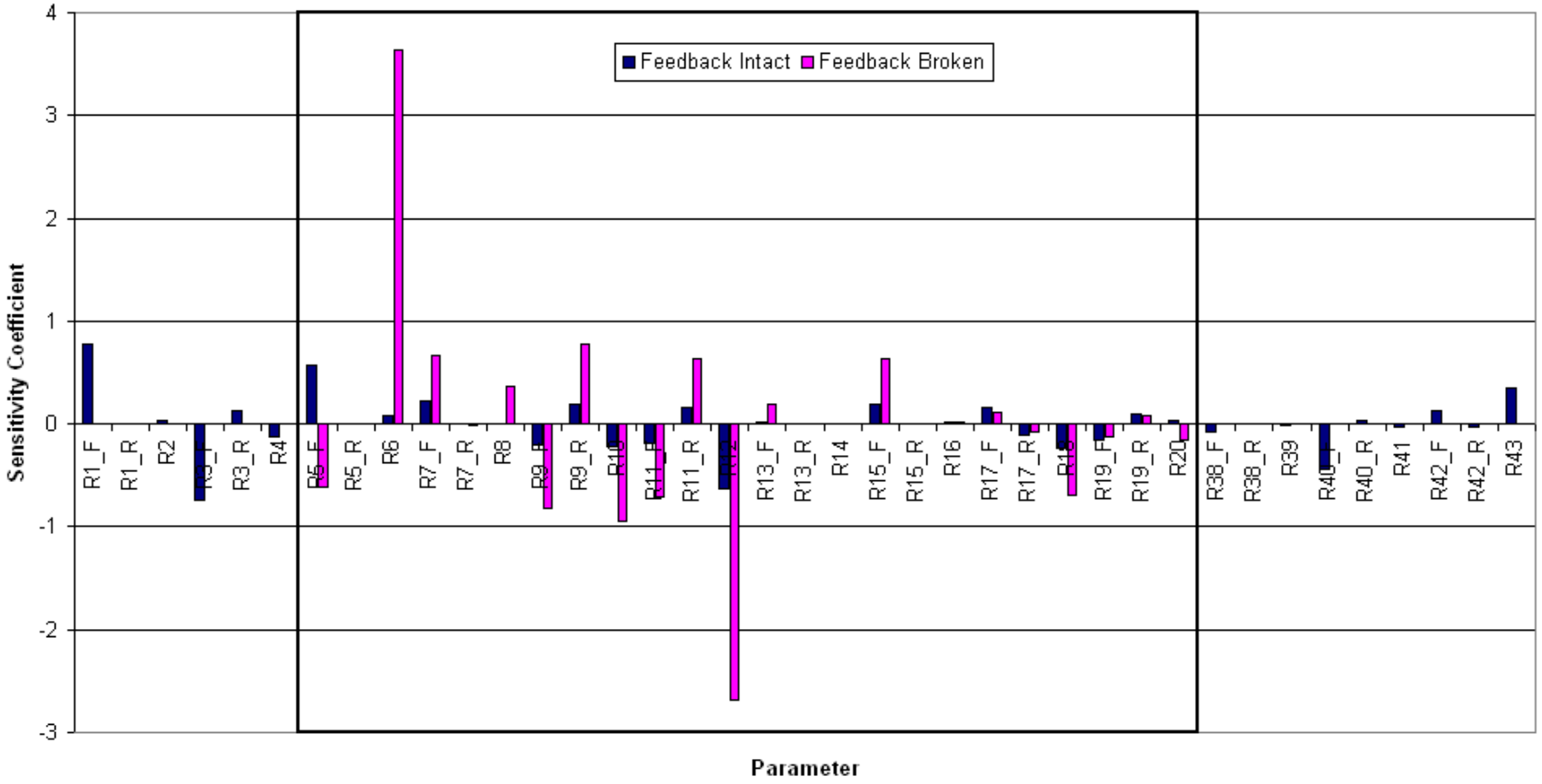




Prediction: Braking the feedback modulates drug response

Computational Modeling 2: Results

Sensitivity of kinetic parameters is decreased due to Negative Feedback



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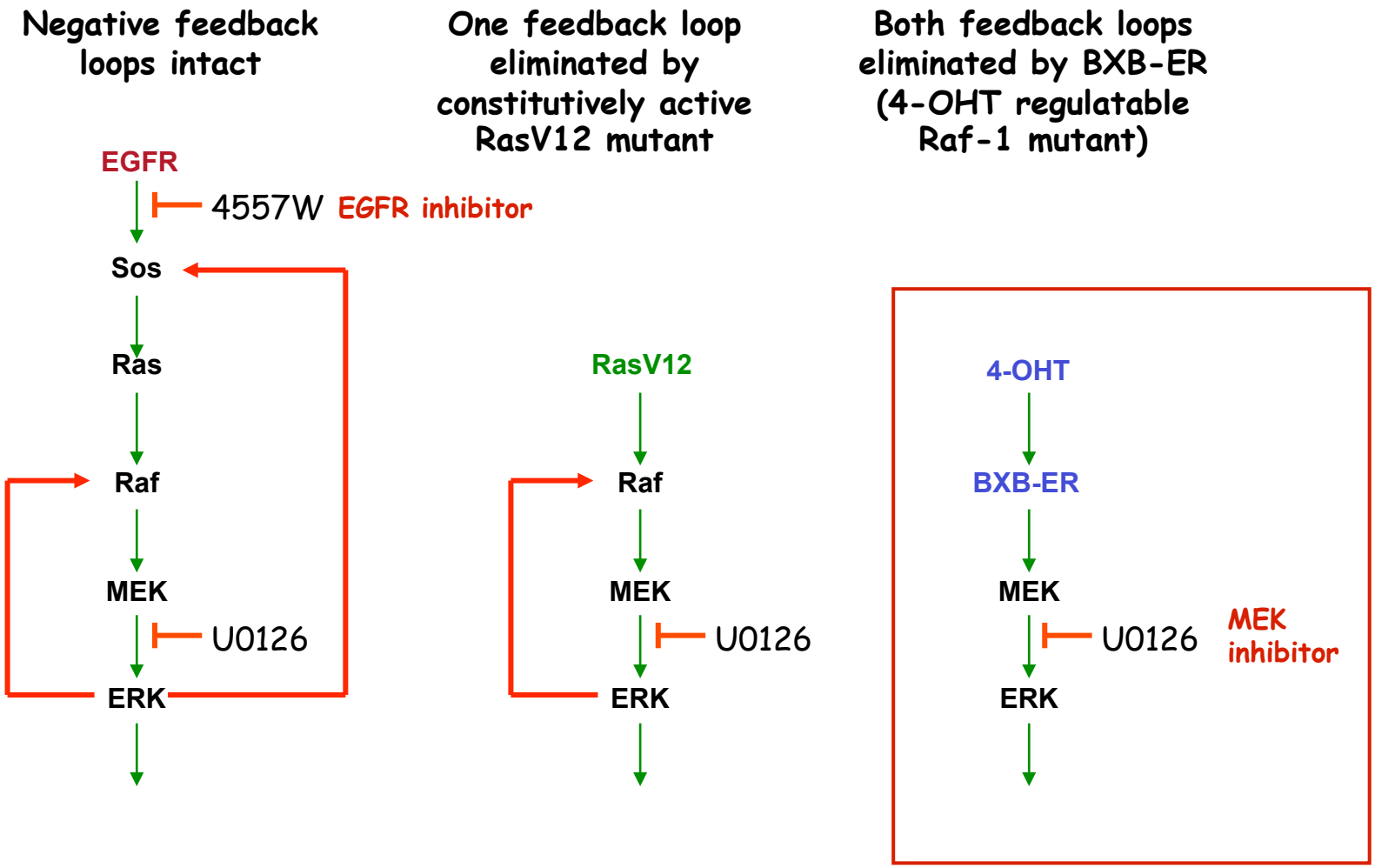
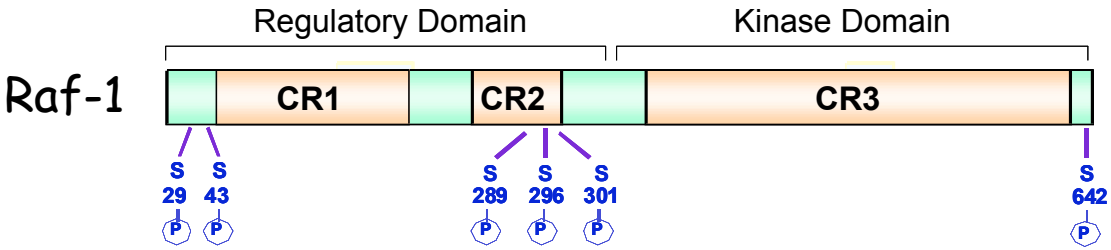
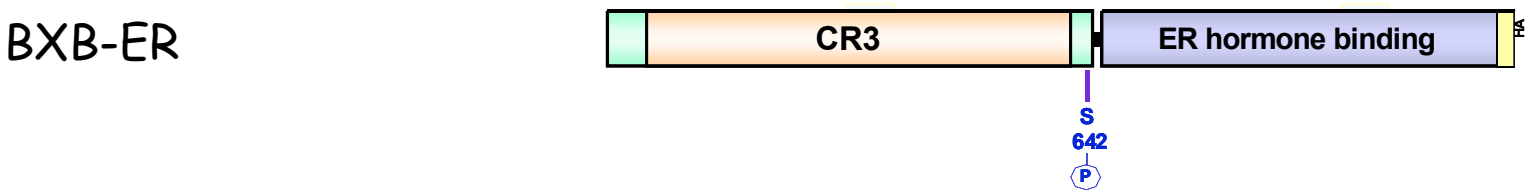
The experimental systems

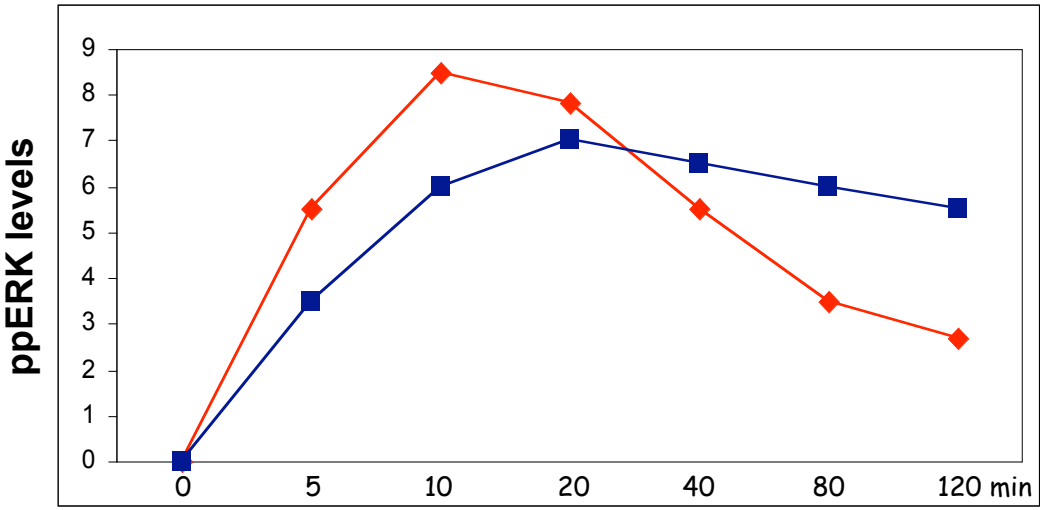


Breaking the ERK feedback with BXBER



ERK feedback phosphorylation sites





BXB-ER stimulated with 4-OHT
(4-Hydroxy Tamoxifen, a synthetic estrogen)

Raf-1 stimulated with EGF

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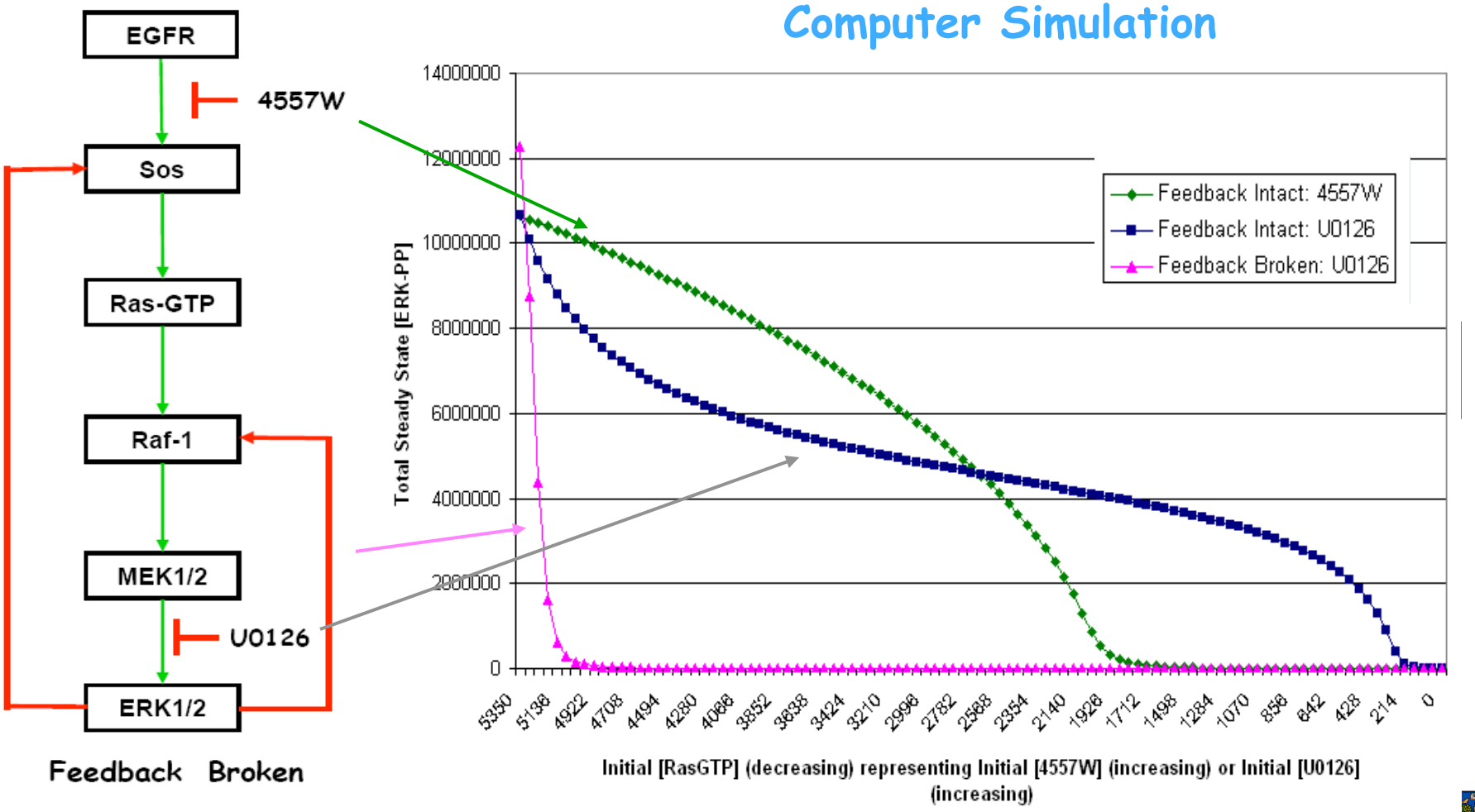
Ablation of feedback by BXBER decreases robustness to MEK-inhibitor U0126



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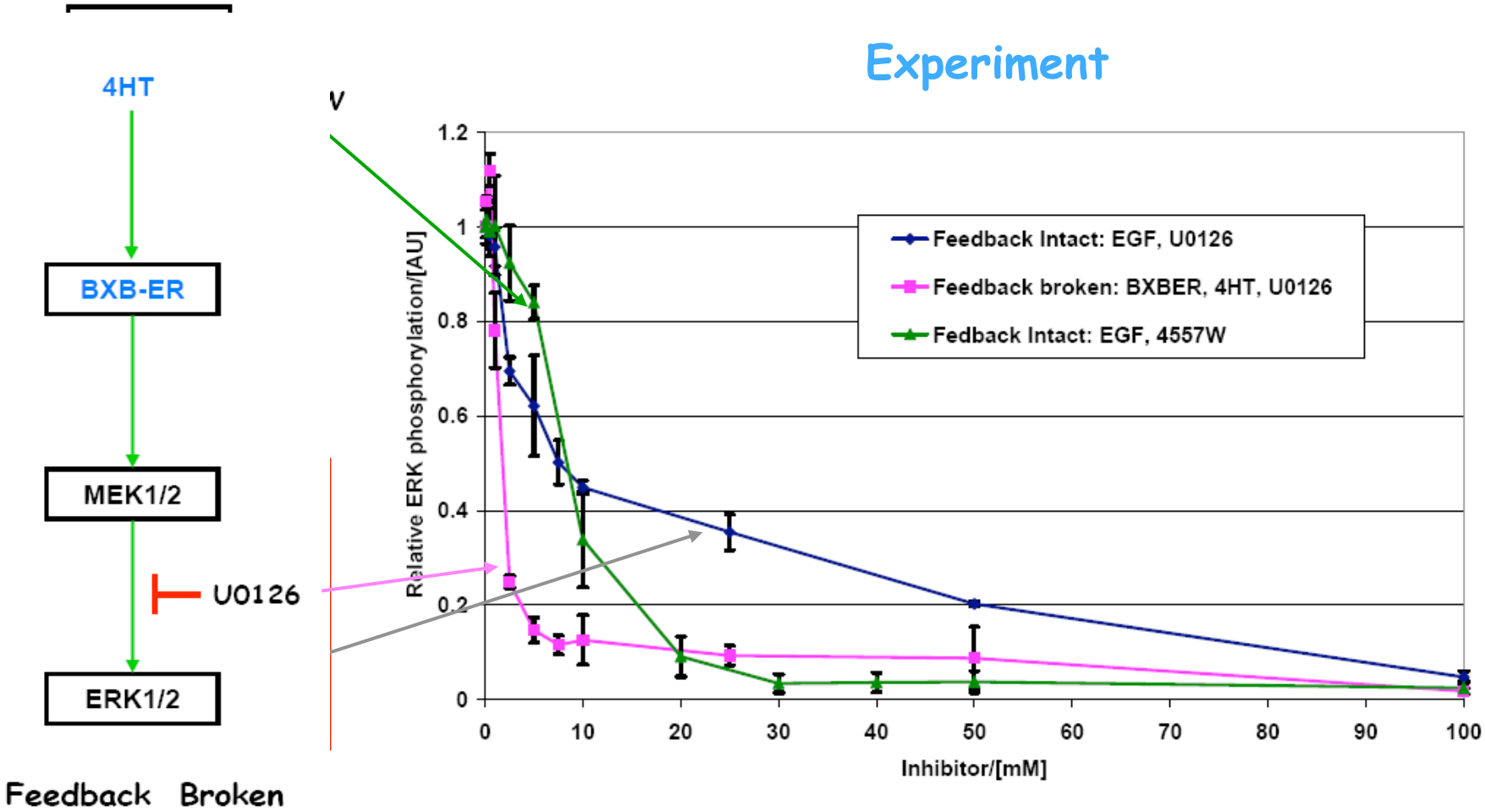
Ablation of feedback by BXBER decreases robustness to MEK-inhibitor U0126



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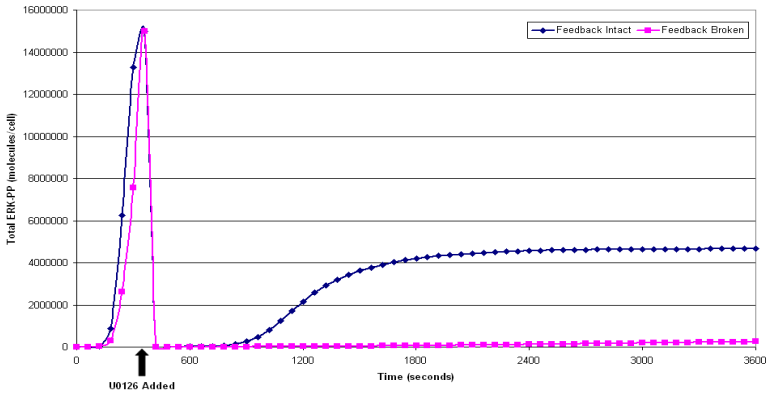
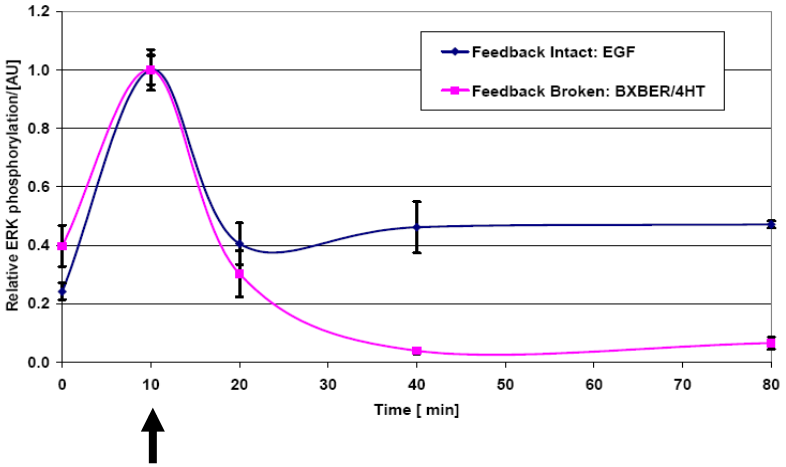
Signal recovery after MEK inhibition

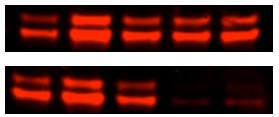
Simulation



Experiment



U0126 added



pERK1/2, +EGF

pERK1/2, + BXBER/4HT

0 10 20 40 80 min stimulation

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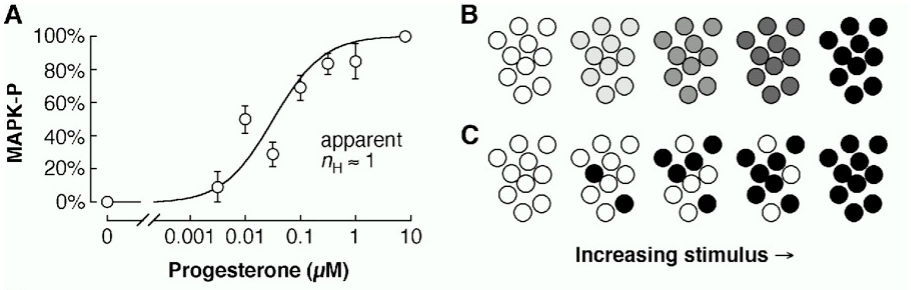
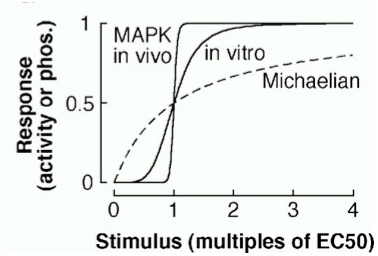
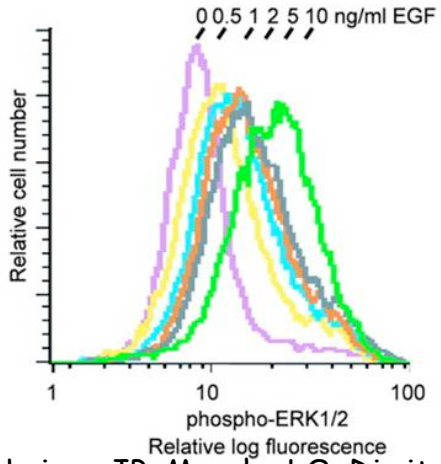
A current dispute: Does ERK activation follow graded or hypersensitive kinetics?

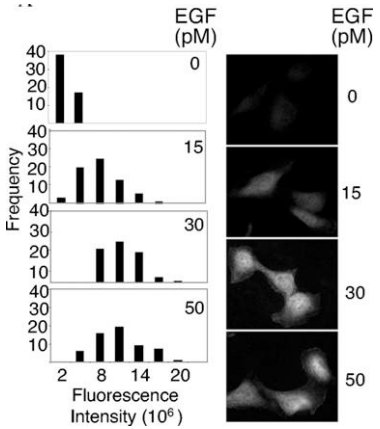




Ferrell JE Jr, Machleder EM.The biochemical basis of an all-or-none cell fate switch in Xenopus oocytes. Science. 1998 May 8;280(5365):895-8

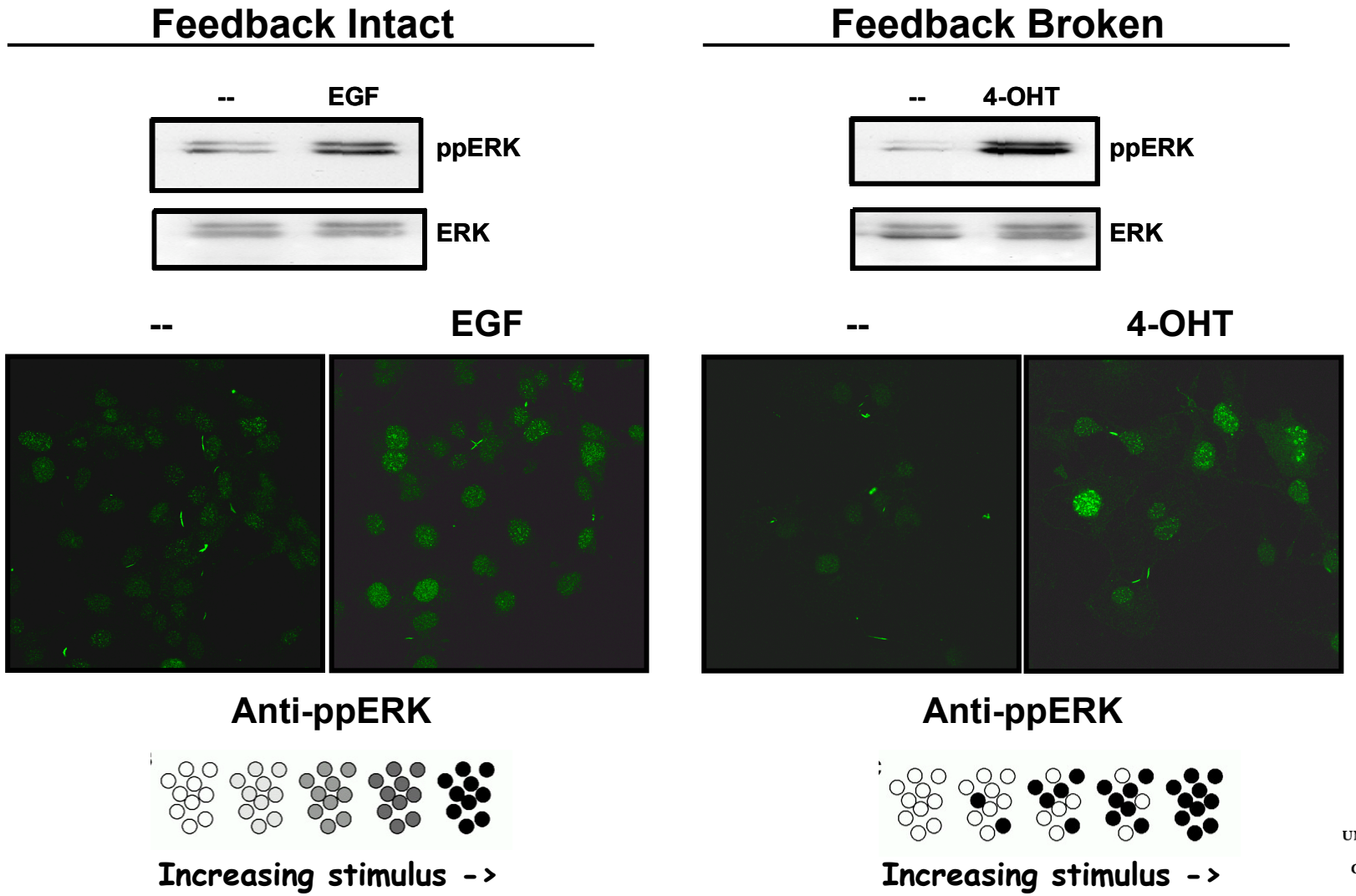


Mackeigan JP, Murphy LO, Dimitri CA, Blenis J. Graded mitogen-activated protein kinase activity precedes switch-like c-Fos induction in mammalian cells. Mol Cell Biol. 2005 Jun;25(11):4676-82.



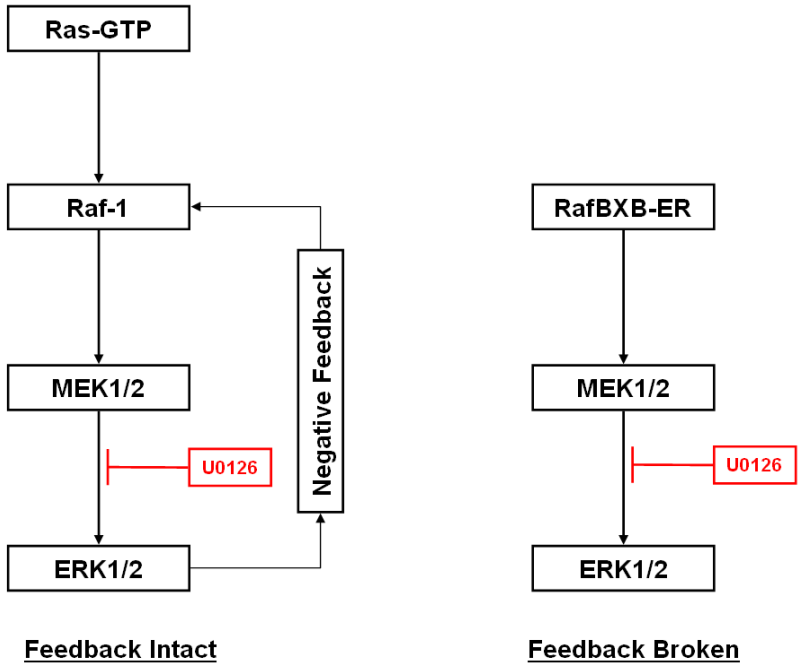
Whitehurst A, Cobb MH, White MA. Stimulus-coupled spatial restriction of extracellular signal-regulated kinase 1/2 activity contributes to the specificity of signal-response pathways. Mol Cell Biol. 2004 Dec; 24(23):10145-50.

Negative Feedback Amplifier could lead to a gradual activation instead of switching – proof remains elusive..



Cancerous Mutations

- The Feedback Intact
  - Ras mutated & always active
  - continual activation of the ERK pathway typically causing cancer
- The Feedback Broken
  - Raf mutated & always active
  - continual activation of the ERK pathway typically causing cancer.
- MEK inhibitors (e.g. U0126) will be effective against cancers caused by Raf mutation (standard amplifier) but ineffective against cancers caused by Ras mutation (negative feedback amplifier)



Cancerous Mutation

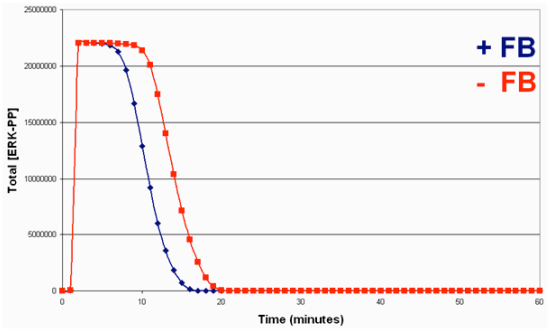
- Solit, 2005 ‘BRAF mutation predicts sensitivity to MEK inihbition’:
  - “The kinase pathway comprising RAS, RAF, MEK and ERK is activated in most human tumours, often through gain-of-function mutations of RAS and RAF family members. Using small-molecule inhibitors of MEK and an integrated genetic and pharmacologic analysis, we find that mutation of BRAF is associated with enhanced and selective sensitivity to MEK inhibition when compared to either ‘wild-type’ cells or cells harbouring a RAS mutation.”
- MEK inhibitors such as U0126 were much more effective in cases of Raf mutations compared to Ras mutations
- However, they did not link this behaviour to the presence of a negative feedback loop or negative feedback amplifier behaviour.

Implications for drug targeting

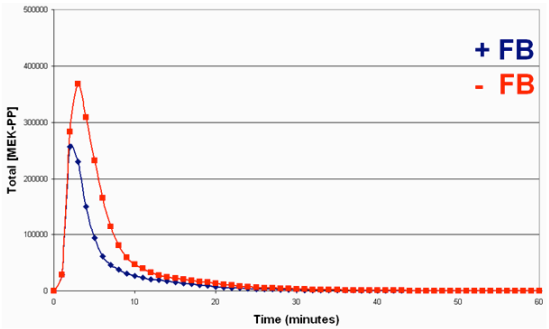
- The aim of a drug is to cause a disruption to the network in such a way that it restores the network to its ‘healthy’ wild-type state.
- Targets must be susceptible to disruption for the drug to have any effect.
- The analysis of feedback suggests that targets inside the feedback loop will prove difficult drug targets because any attempt to disturb these targets will be resisted by the feedback loop.
- Wet-lab results show clear negative feedback amplifer characteristics

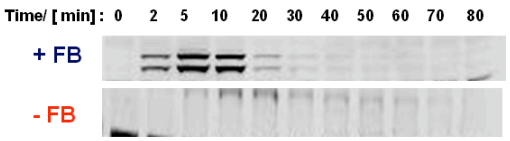
Redundancy in the EGFR/ERK signalling pathway

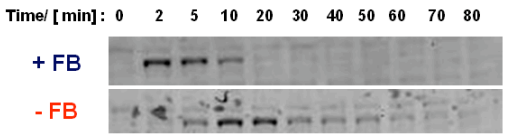
ERK-PP

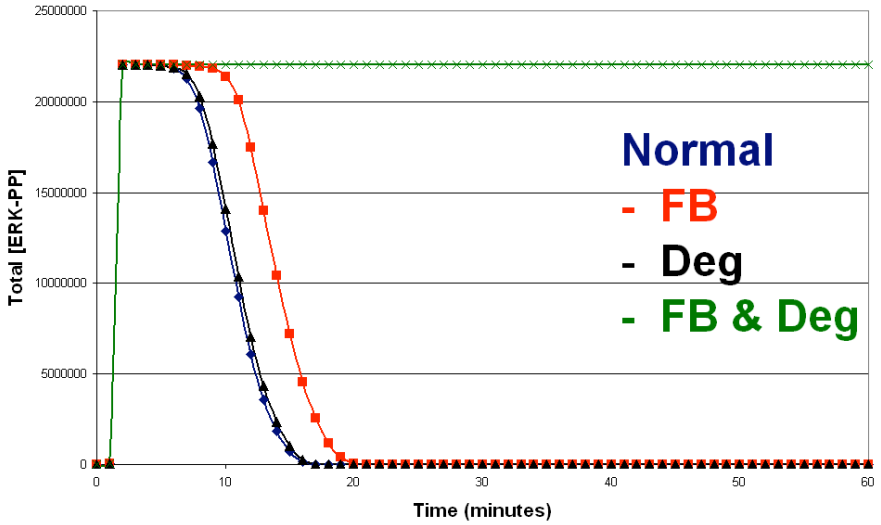


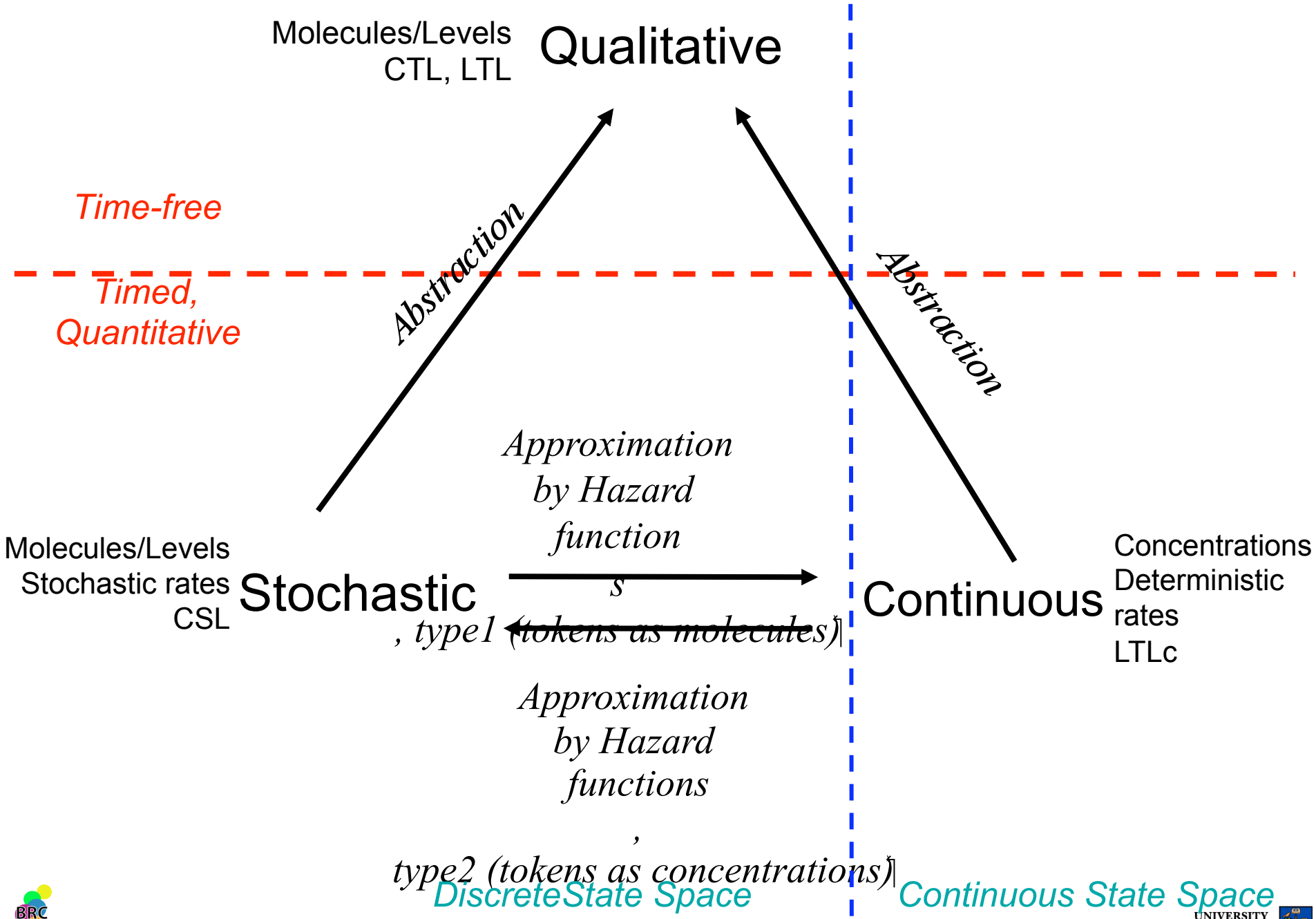
MEK-PP










Molecules/Levels
CTL, LTL
Qualitative
Time-free
Timed,
Quantitative
Abstraction
Abstraction
Approximation
by Hazard
function
s
Molecules/Levels
Stochastic rates
CSL
Stochastic
, type1 (tokens as molecules)
Continuous
Concentrations
Deterministic
rates
LTLc
Approximation
by Hazard
functions
,
type2 (tokens as concentrations)
DiscreteState Space
Continuous State Space

Hazard functions

- Hazard function type1 (tokens as molecules)

$$h_t := c_t \cdot \prod_{p \in \bullet t} \binom{m(p)}{f(p,t)}$$

- c_t transition specific stochastic rate constant
- m(p) current number of tokens on pre-place p of transition t
- binomial coefficient number of non-ordered combinations of the f(p,t) molecules, required for the reaction, out of the m(p) available ones.

- Hazard function type2 (tokens as concentrations)

$$h_t := k_t \cdot N \cdot \prod_{p \in \bullet t} \left(\frac{m(p)}{N} \right)$$

- k_t transition deterministic rate constant
- N number of levels
- Levels: Calder et al, Trans Comp Sys Bio VI, LNBI 4220, 2006

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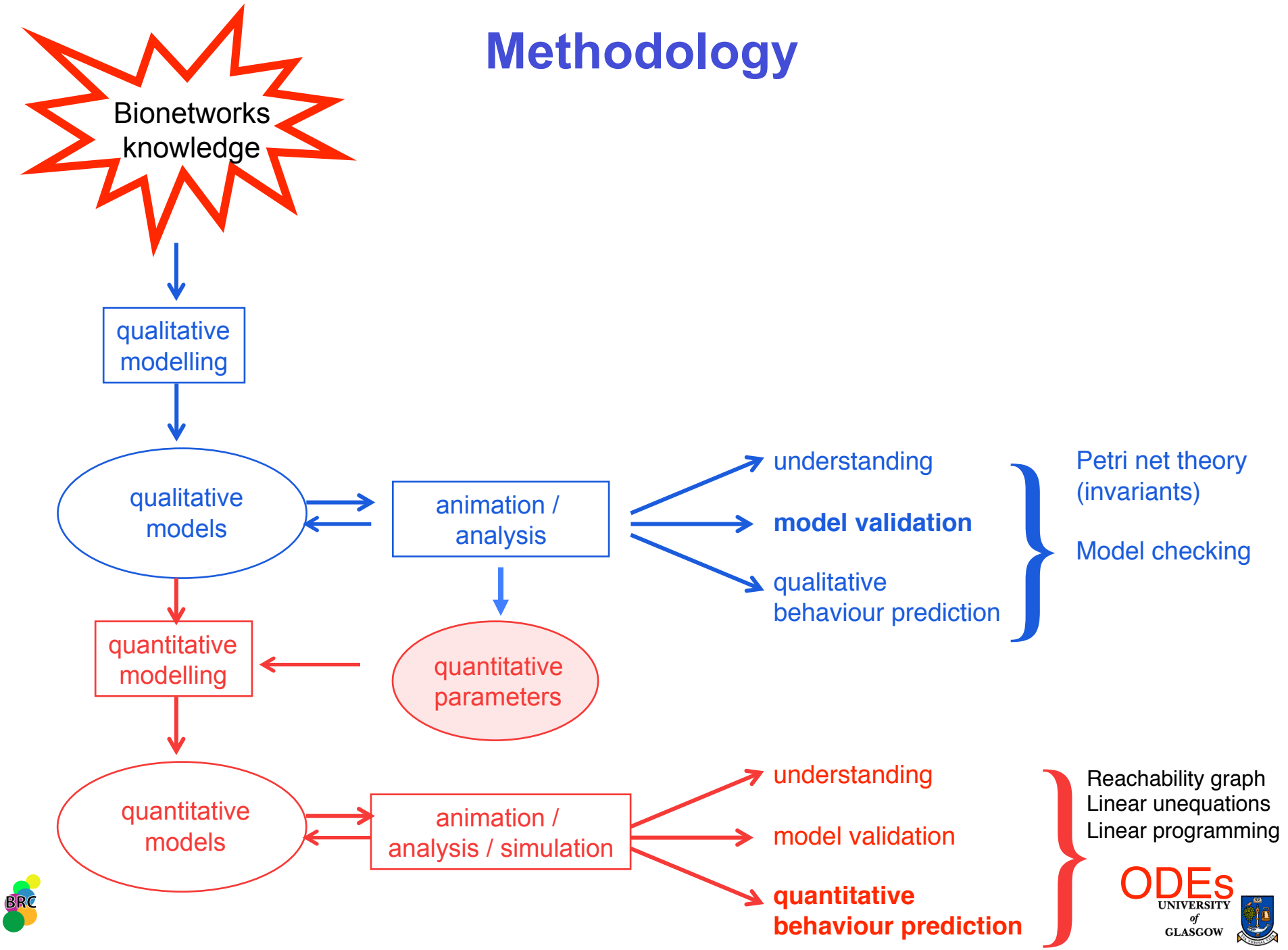
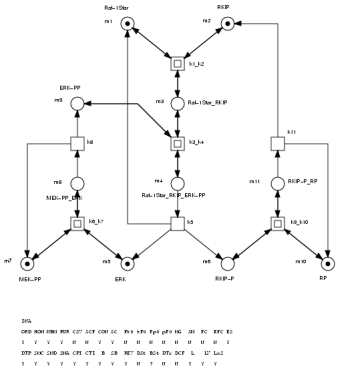
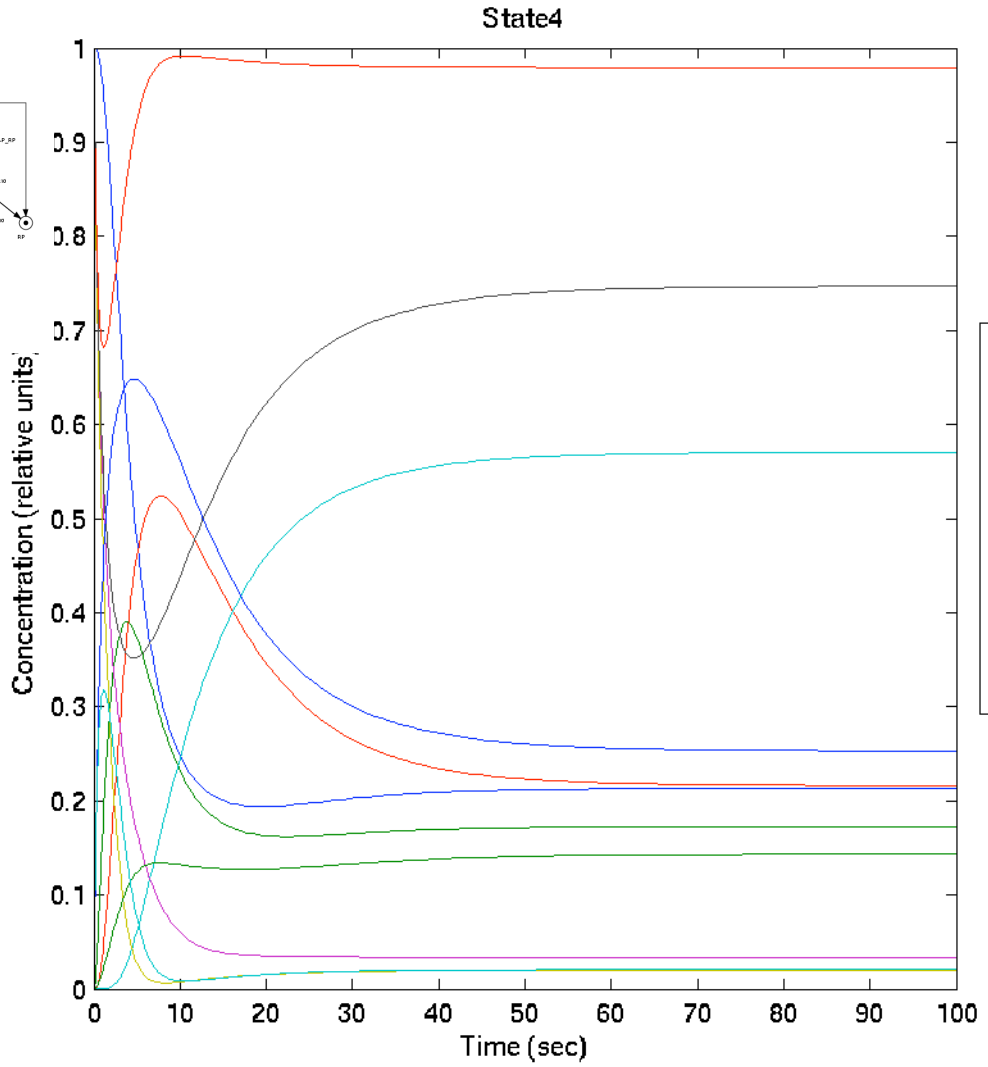
Methodology



From Petri Nets to Differential Equations - an Integrative Approach

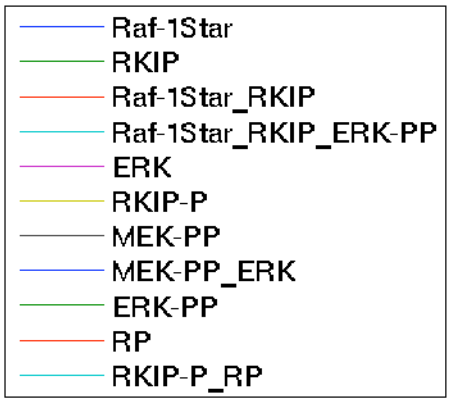
David Gilbert & Monika Heiner

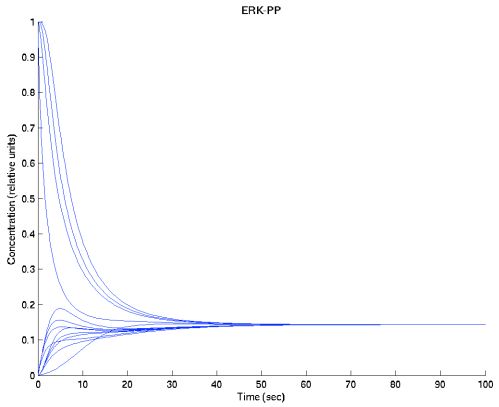




Species	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Raf-1*	1	0	0	1	1	1	1	1	0	0	1	1	1
RKIP	1	0	0	0	0	0	0	1	0	0	1	0	0
Raf-1*	0	1	0	0	0	0	0	0	1	1	0	0	0
Raf-1*/RKIP/ERK-PP	0	0	1	0	0	0	0	0	0	0	0	0	0
ERK	0	0	0	1	0	0	1	1	1	0	0	0	0
RKIP-P	0	0	0	1	1	0	0	0	0	0	0	0	1
MEK-PP	1	1	1	1	0	0	1	1	1	0	0	1	1
MEK-PP/ERK	0	0	0	0	1	1	0	0	0	1	1	0	0
ERK-PP	1	1	0	0	0	0	0	0	0	0	0	1	1
RP	1	1	1	1	1	0	0	1	1	1	1	0	1
RKIP-P/RP	0	0	0	0	0	1	1	0	0	0	0	1	0

Initial 13 'good' state configurations





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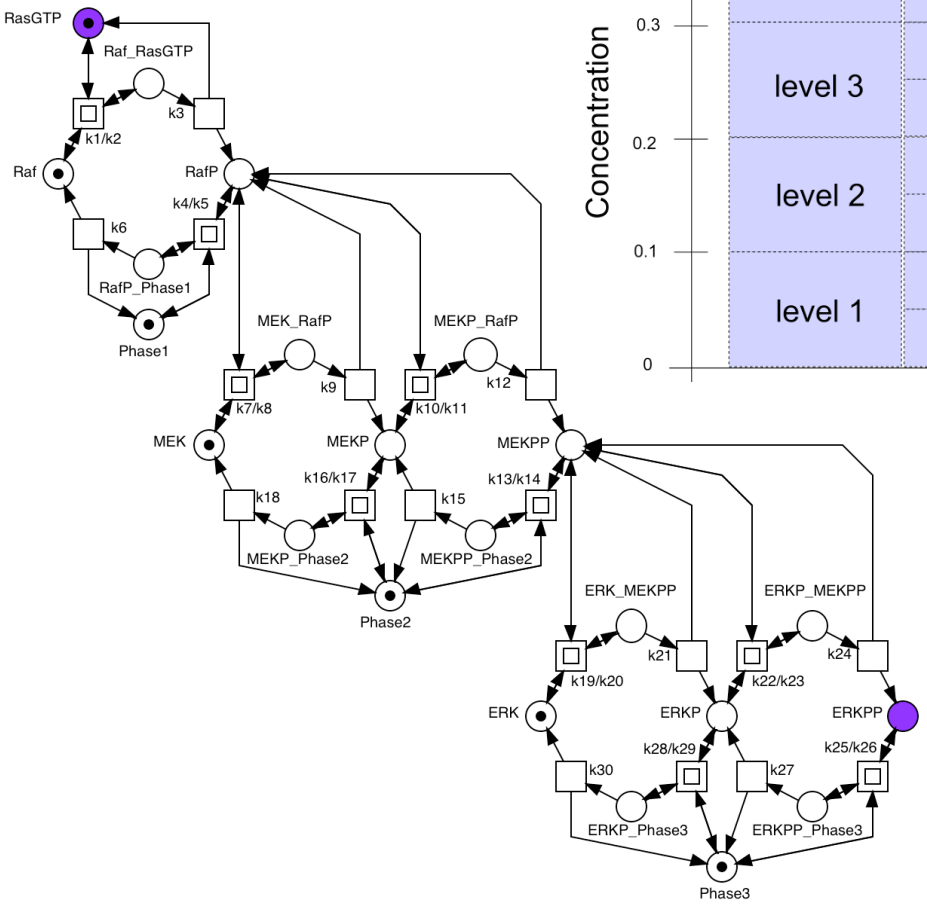
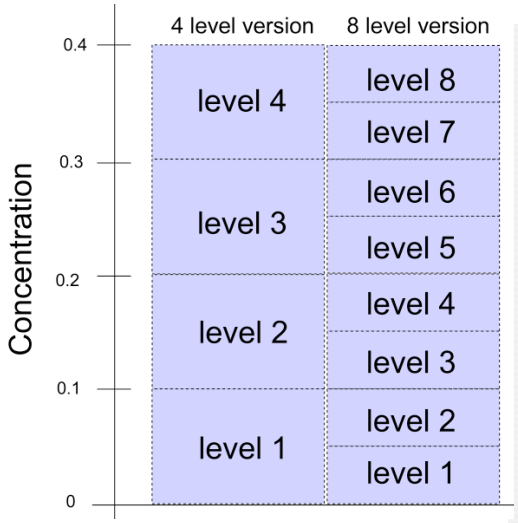
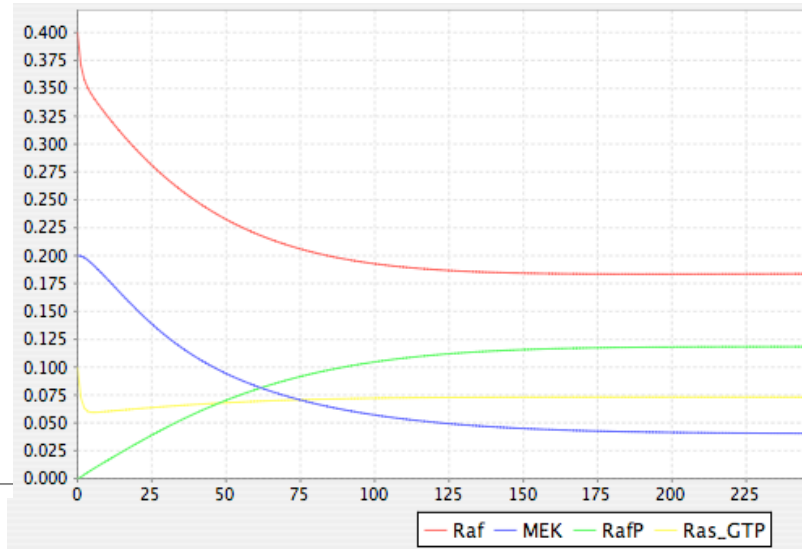
Levchenko model







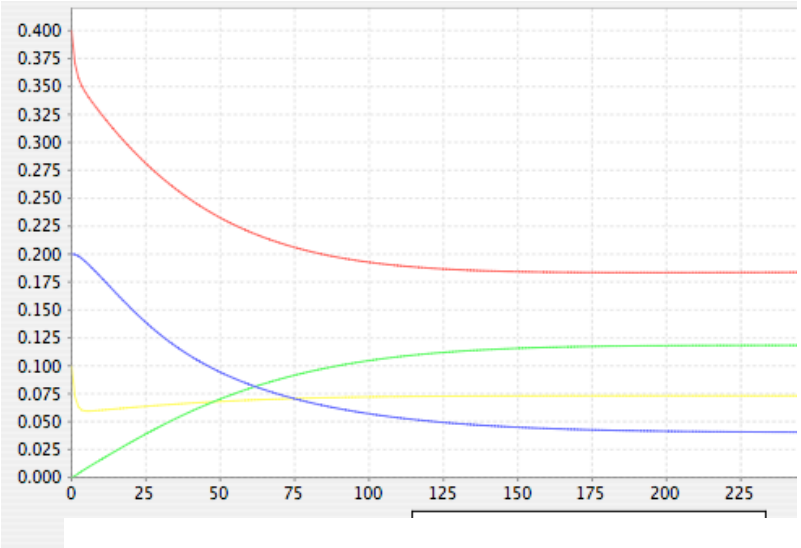
Levchenko et al, PNAS, 2000

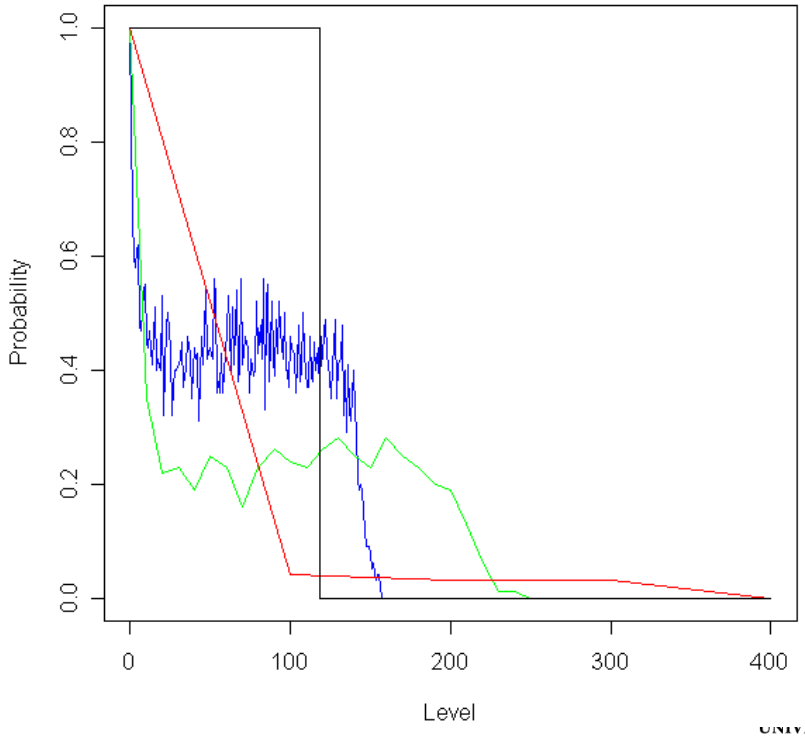
Calder et al, CMSB 2005, TCSB 2006

Continuous Stochastic Logic model checking

- Property S1: What is the probability of the concentration of RafP increasing, when starting in a state where the level is already at K?

$$P_{=?}\ [\ (RafP = L)\ U\ (RafP > L)\ \{RafP = L\}]$$





- *Stochastic: 4 (red), 40 (green) 400 (blue) levels*
- *Extensible to thousands*
- *Approximates to deterministic behaviour (**black**) 0.1182...*

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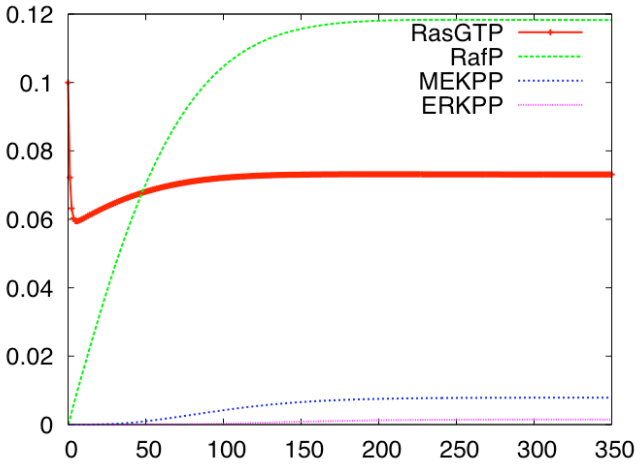
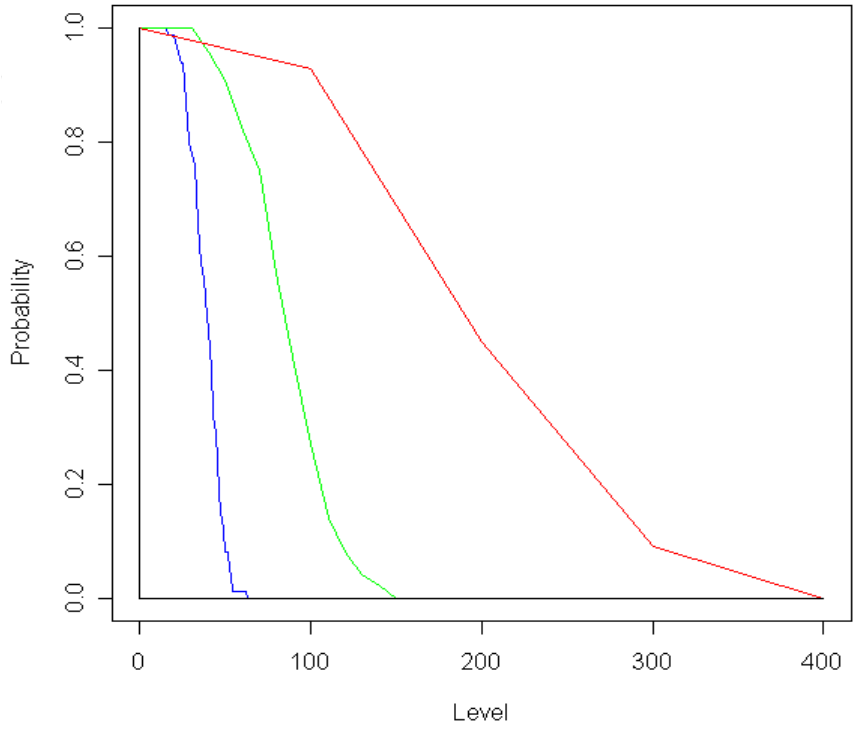
CSL model checking

- Property S2: What is the probability RafP being the first species to react?

$$P_{=?}\ [\ ((MEKPP = 0) \wedge (ERKPP = 0))\ U\ (RafP > L)\ \{(MEKPP = 0) \wedge (ERKPP = 0) \wedge (RafP = 0)\}$$





- *Stochastic: 4 (red), 40 (green) 400 (blue) levels*
- *Extensible to thousands*

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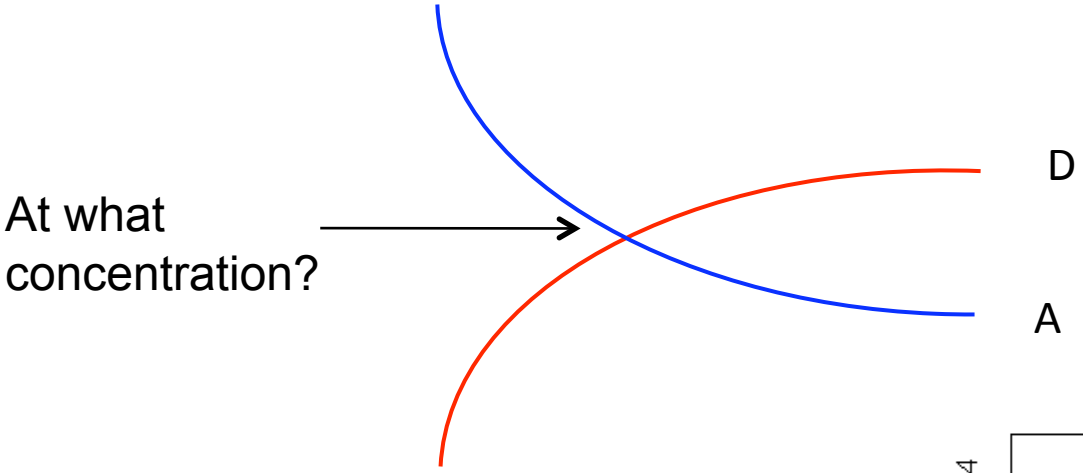


Two reactions:
(1) A->B
(2) C->D

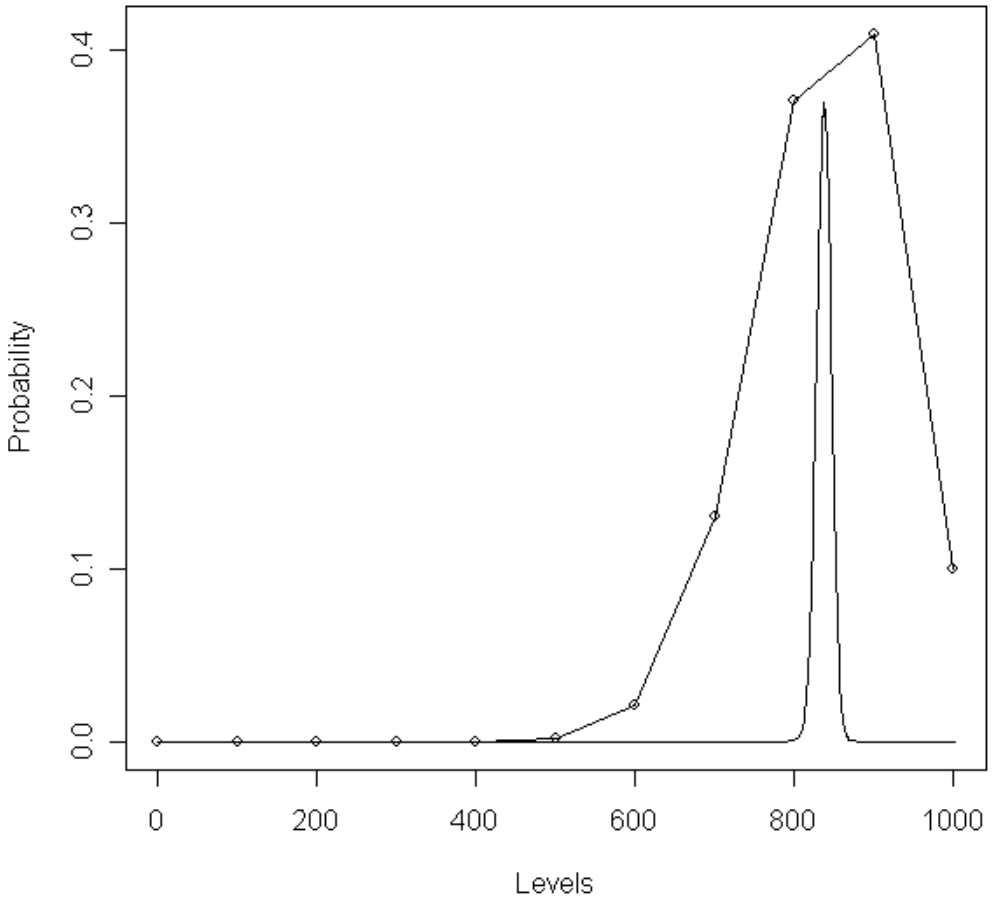
Property: P=?[([A] = X) { [A]=[D] }]

Assessing [X] at which reactant [A] equals product [D]

Results using 10 and 1,000 levels.

1,000 levels: peaks at 837, I.e. 8.37 the most probable concentration when [A] = [D]

ODE simulation; [A] = [D] at concentration ~8.35



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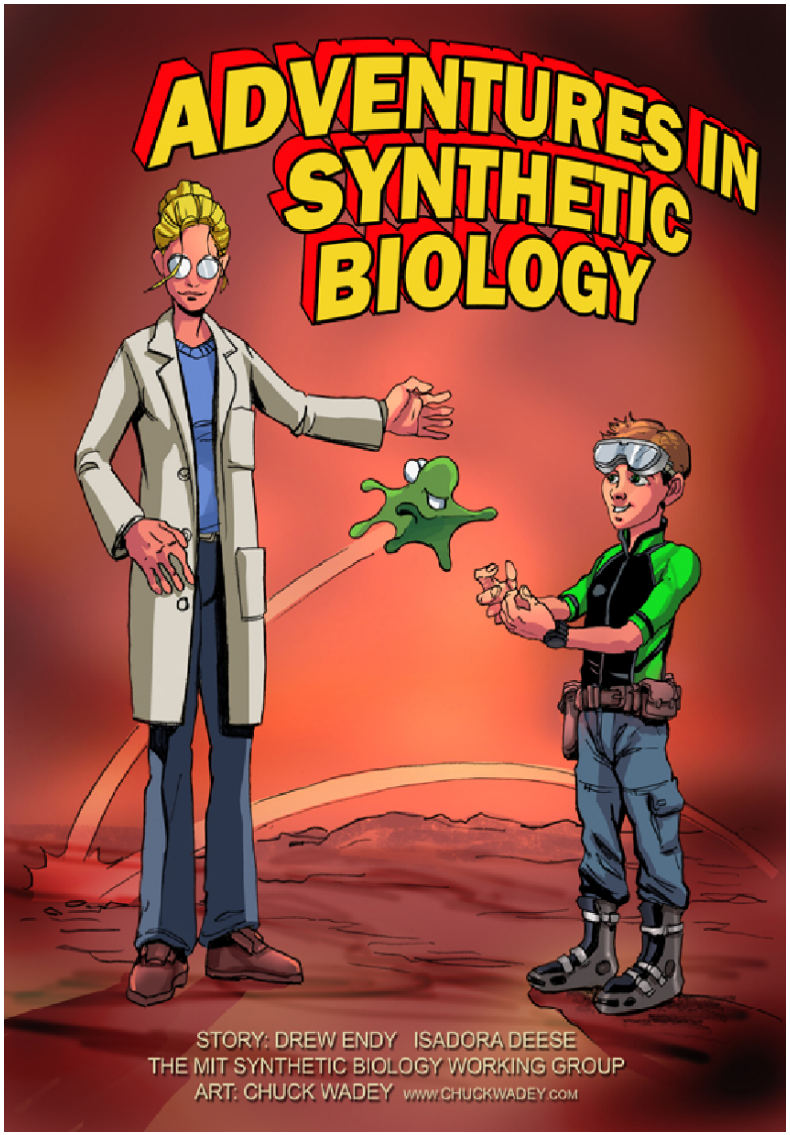
What is synthetic biology?



- Design & construction of new biological parts, devices, and systems
- Re-design of existing, natural biological systems for useful purposes
- Involves
  - *Standardisation*
  - *Decoupling*
  - *Abstraction*

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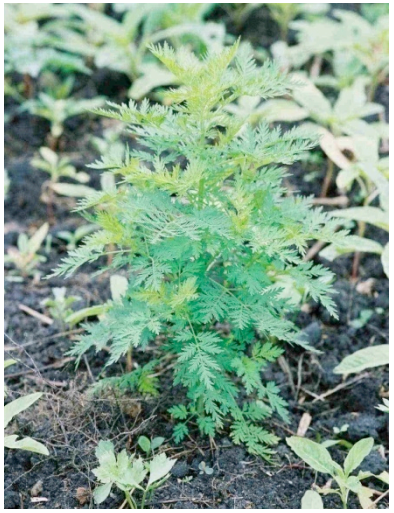
A drug manufacturing plant

- “Audacious plan” New Scientist, May 2006
- Engineer e.coli / yeast to synthesise the anti-malarial artemisinin
- $42.6 million, Bill & Melinda Gates Foundation



Artemisia annua

- Plant difficult to grow and only yield minute quantities of drug per kilo
- Artemisinin is expensive

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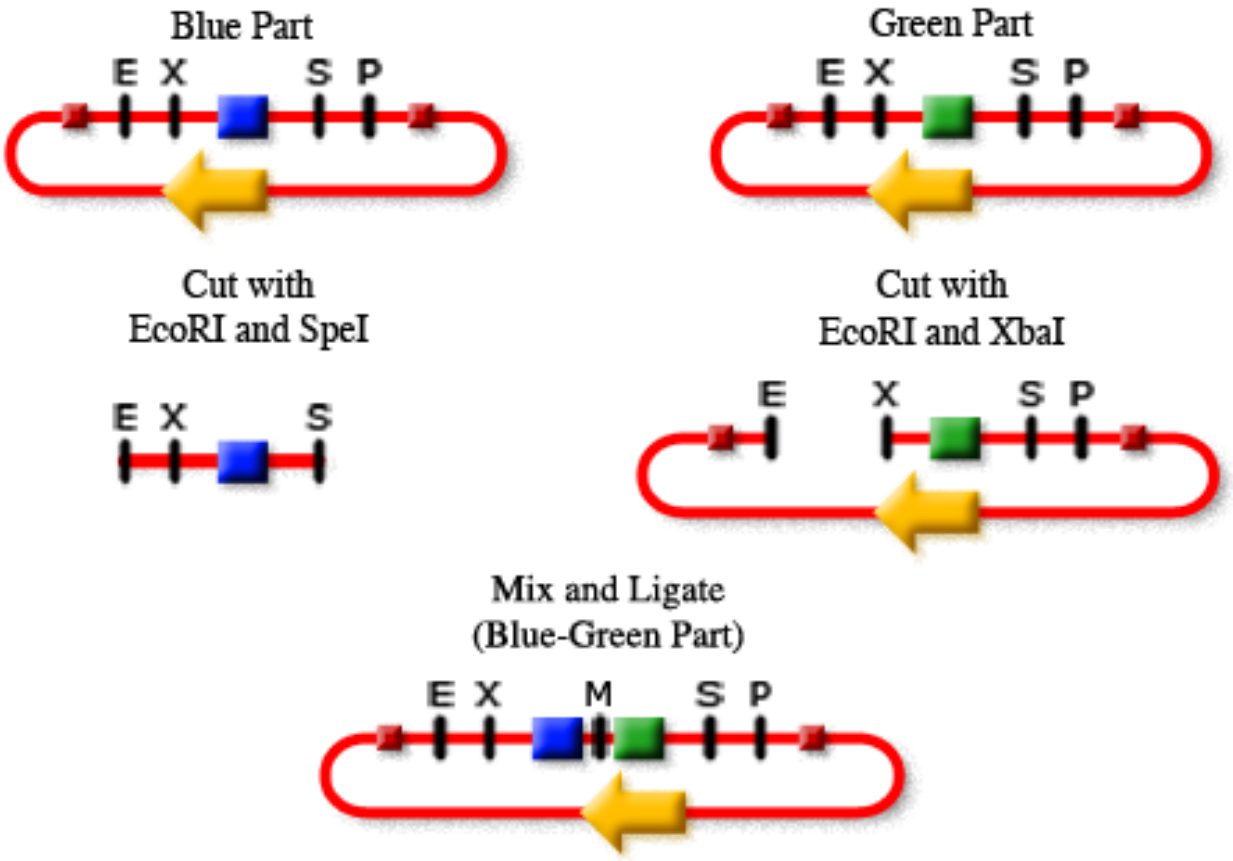
BioBrick Parts Assembly Strategy



The Registry

Systems

- Measurement
- Measurement (Under Development)
- Projects(empty)

Devices

- Reporters
- Inverters
- Signalling
- Protein Generator
- Composite Devices
- Measurement

Parts

- Ribosome Binding Sites
- Regulatory
- RNA
- DNA
- Protein Coding
- Terminators
- Conjugation

Chassis

- E.coli Strains

Mammalian

Vectors

- Plasmids

Other

- Yeast Parts
- Construction Intermediate
- PCR Primer
- Tags
- Other
- Deleted
- Bacteriophage T7

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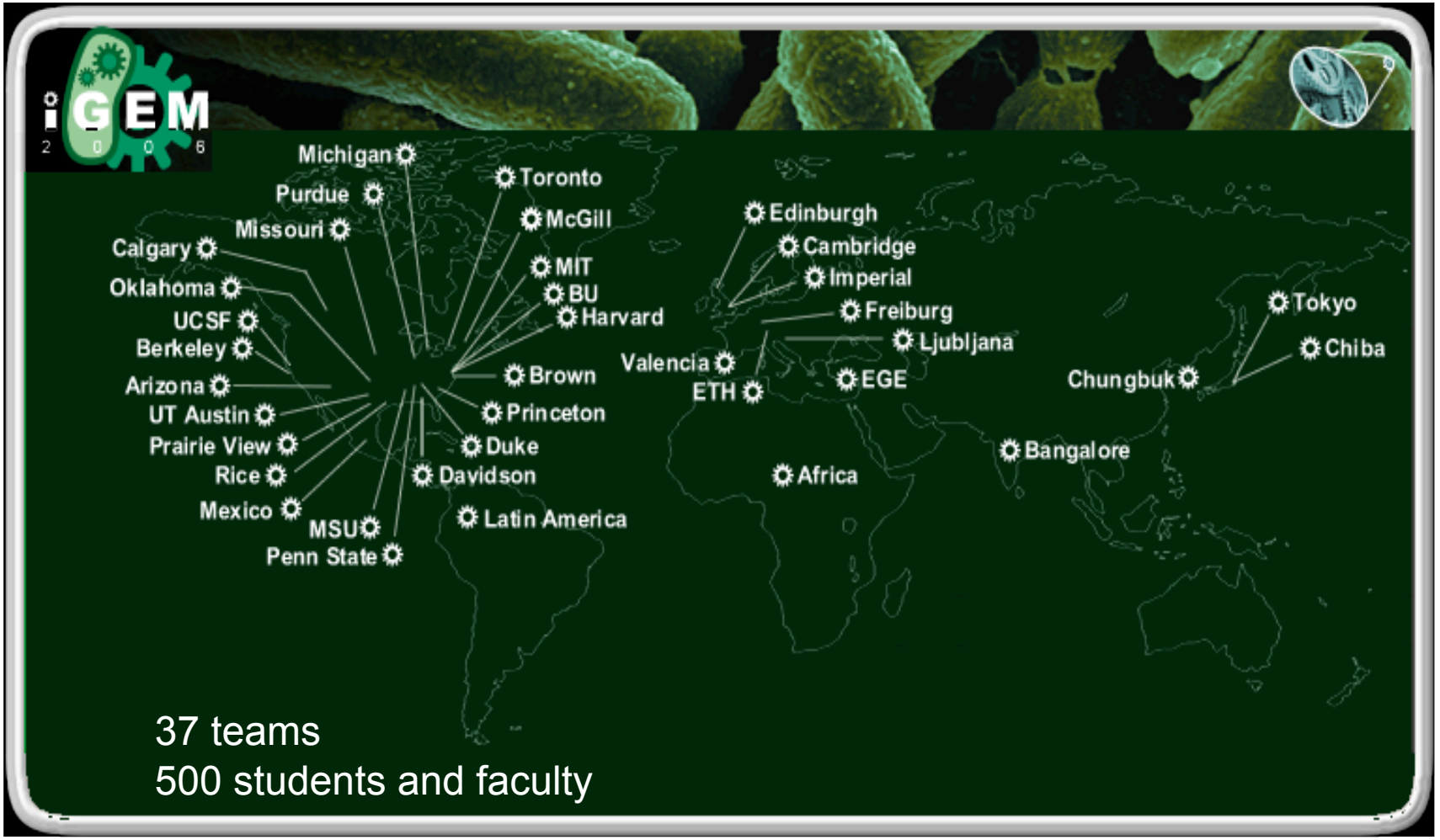
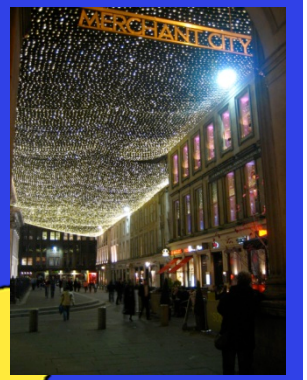


2007: 54 teams, 750 students & faculty



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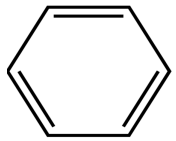
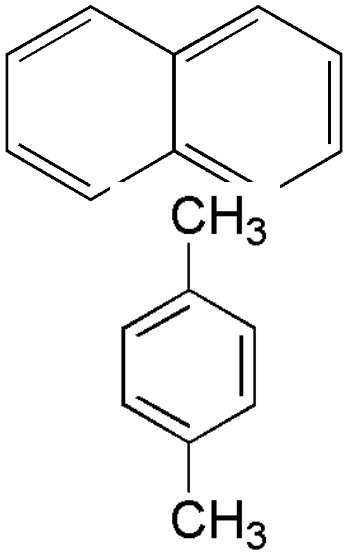
The Problem



Phenolic compounds



Polycyclic aromatic hydrocarbons (PAH)

BTEX compounds

Why a Biosensor?

- Lab-based monitoring
- Skilled workforce
- Expensive!

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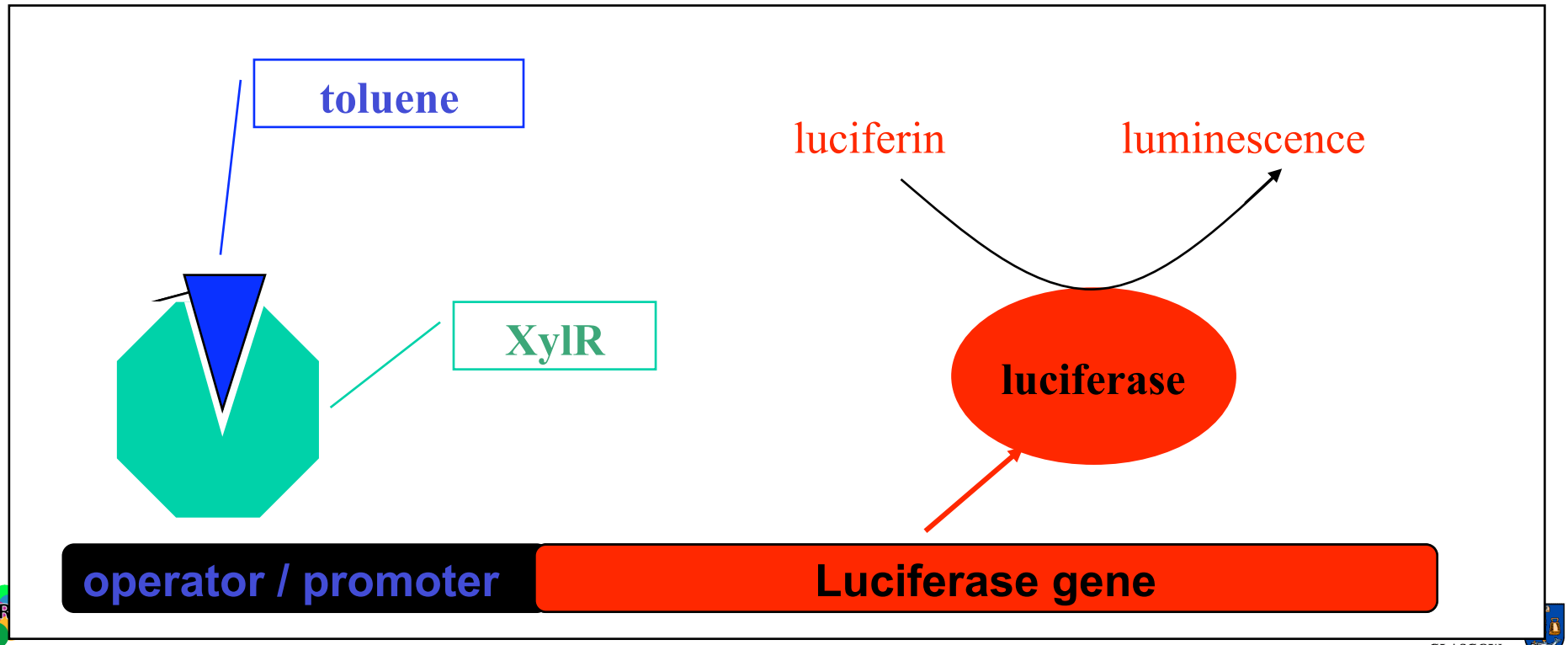
What is a Biosensor?

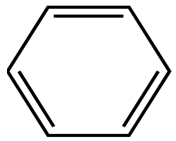
- Biosensors include a transcriptional activator coupled to a reporter



Objectives

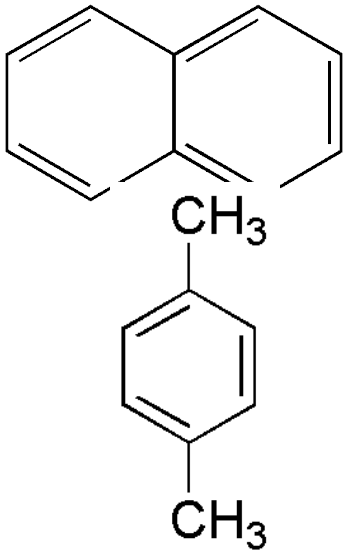
- 1: Design modular sensor construct
  - Switch on reporter in presence of pollutants
- 2: Create the construct
- 3: Test the system
- 4: Development into a machine
- 5: Model and predict outcomes!

Our Solution



Phenolic compounds

DmpR - phenols



Polycyclic aromatic hydrocarbons (PAH)

DntR - PAHs

BTEX compounds

XylR - toluene

Our Construct Design

Constitutive promoter

RBS

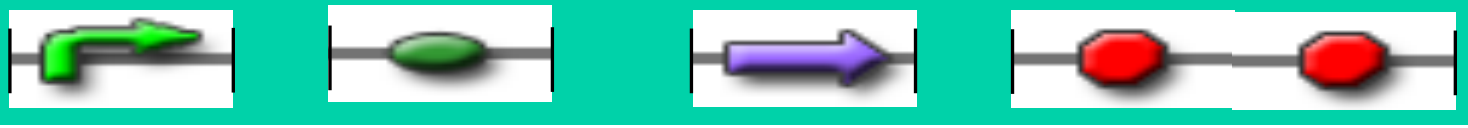
Transcriptional activator

DmpR - phenols

DntR - PAHs

XylR - toluene

Double terminator BBa_B0015



Responsive promoter

RBS BBa_J61101

Reporter gene

LacZ

GFP

Luciferase

Double terminator BBa_B0015

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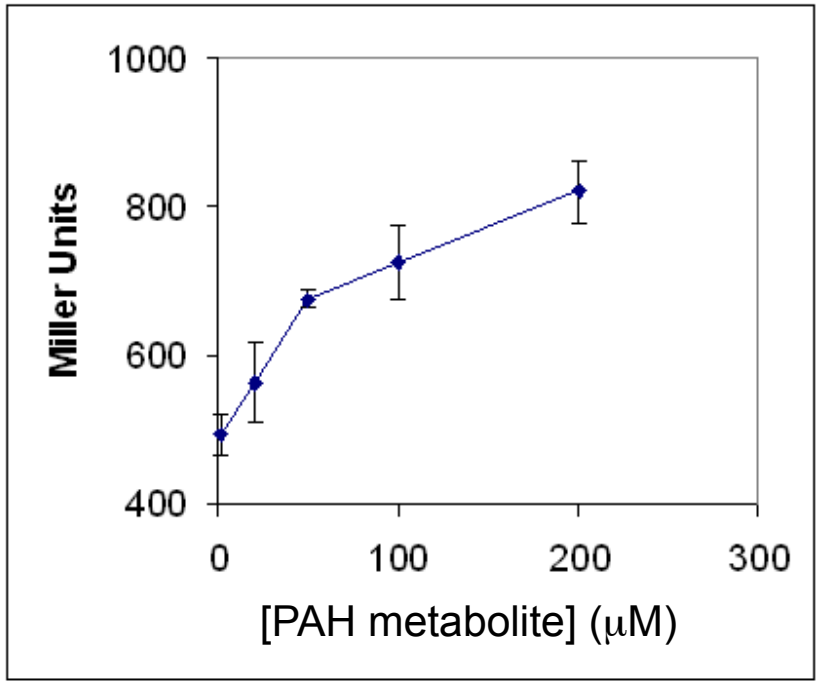
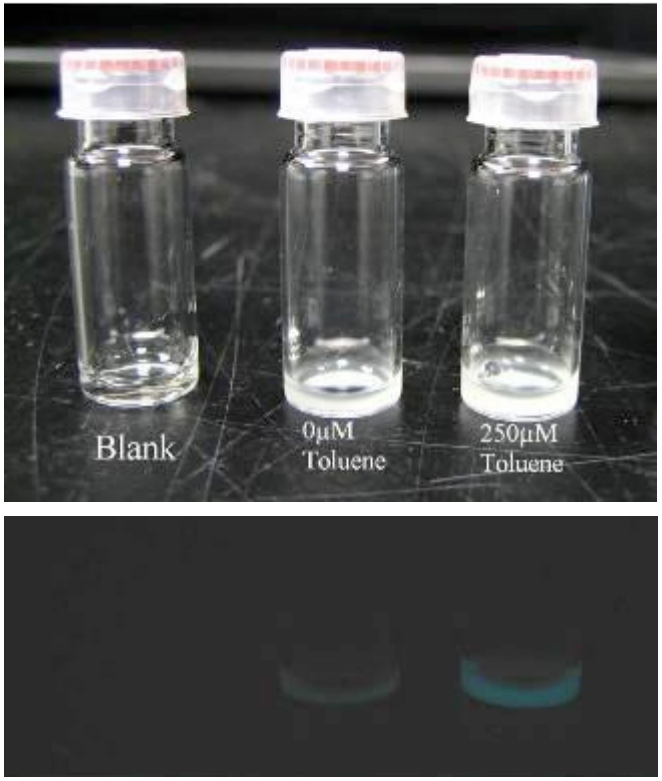
Testing The System

DntR - inducible LacZ

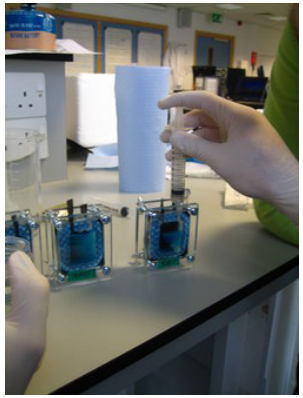


XylR - inducible luciferase



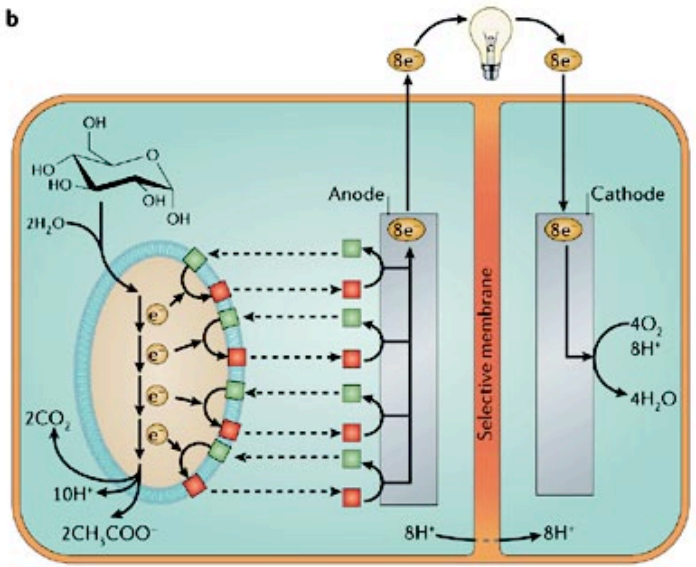
Unique Reporter System

- 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

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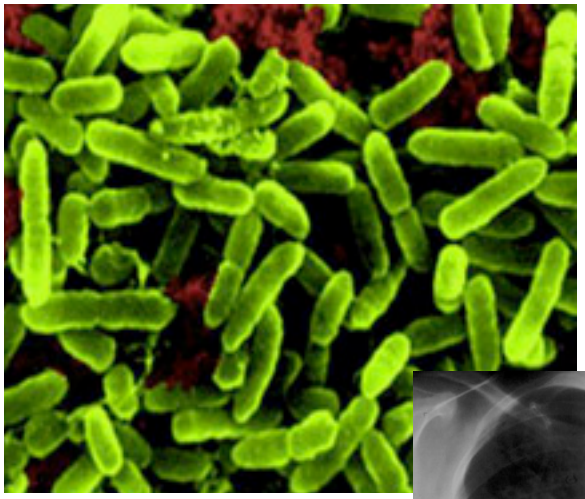
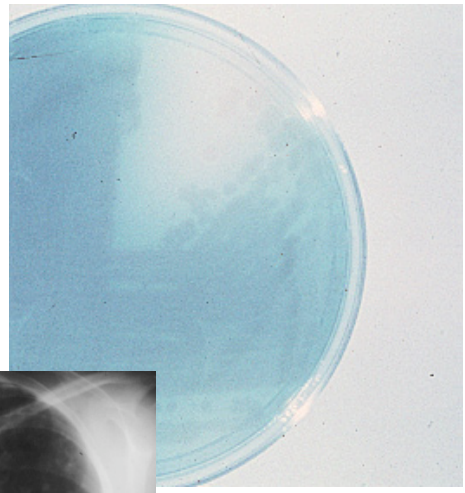
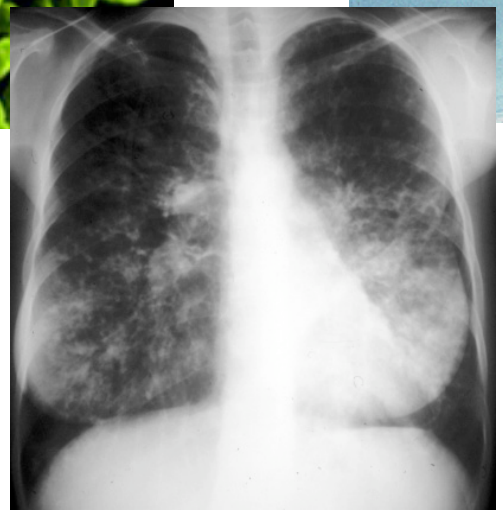
Pyocyanin

- From pathogenic *Pseudomonas aeruginosa*
- Can act as electron mediator



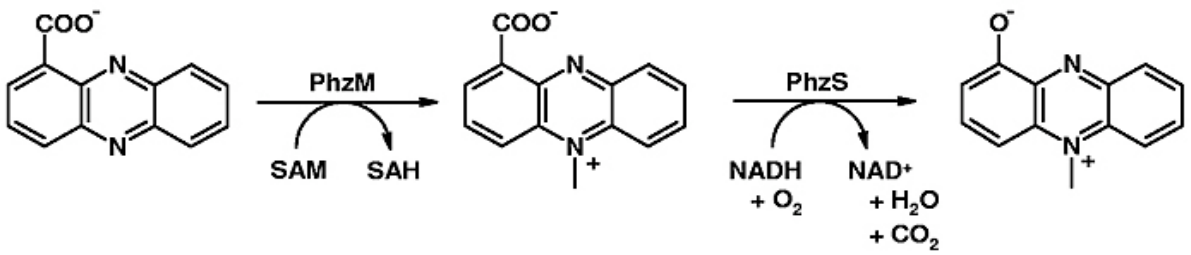




Pyocyanin

- Phz genes – 7 gene operon, pseudomonad specific

- PhzM and PhzS – *P. aeruginosa* specific



PCA

Pyocyanin

Biosynthesis of pyocyanin

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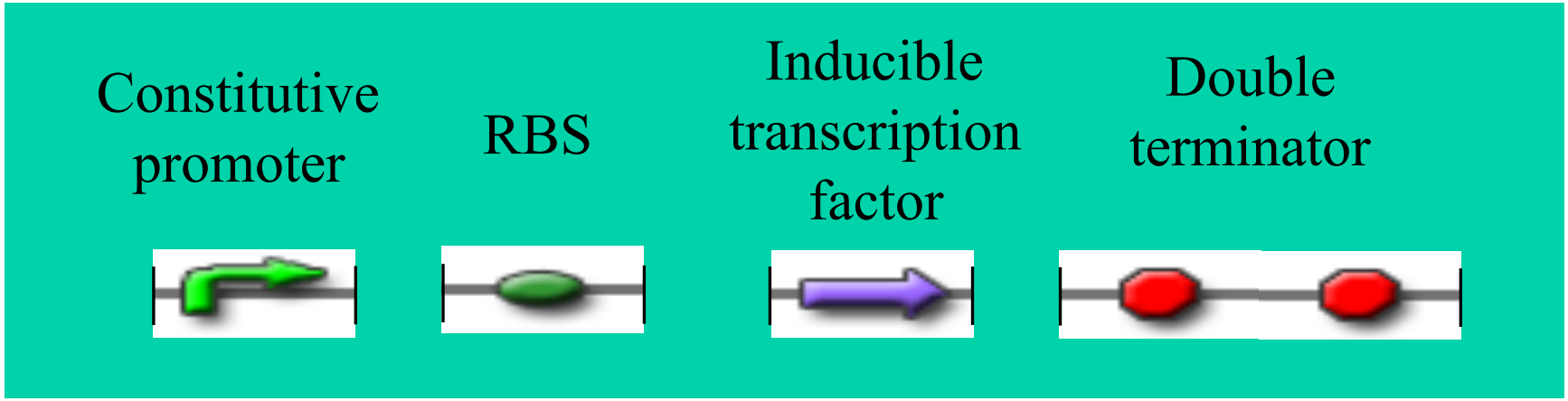
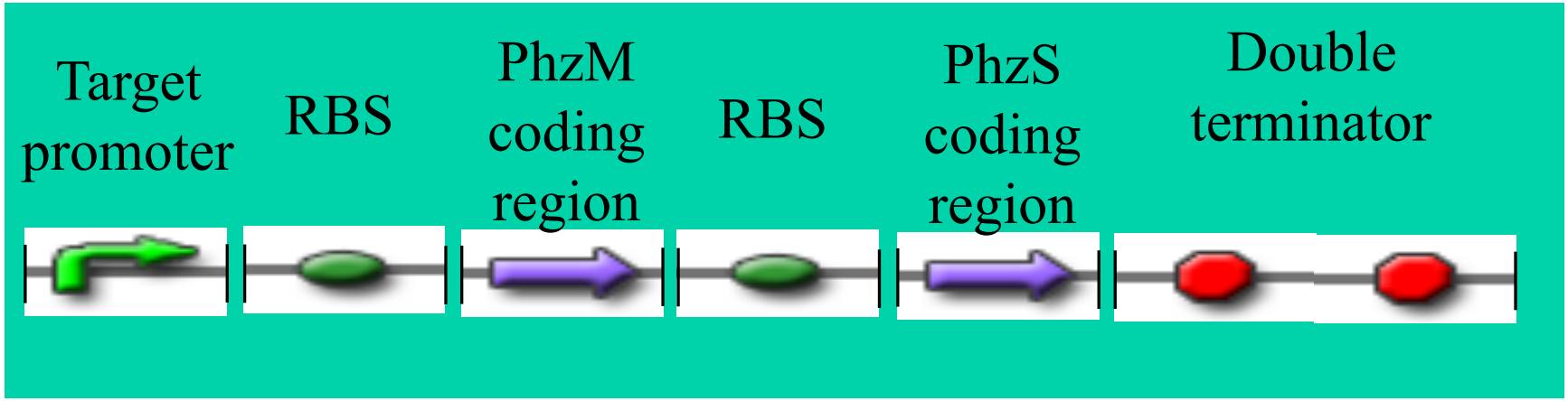
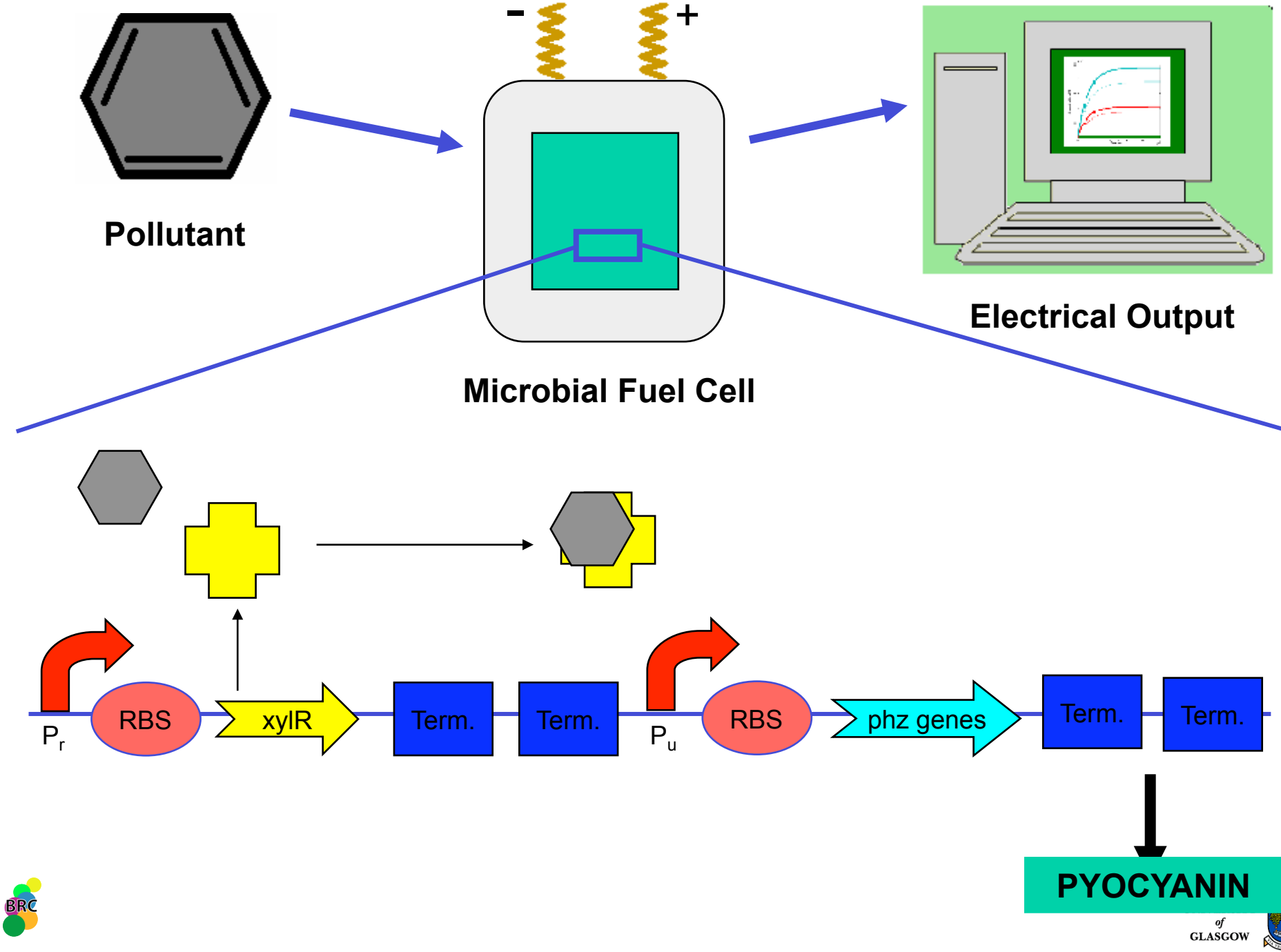
Our Constructs






Pollutant
-
+
Electrical Output
Microbial Fuel Cell
P r
RBS
xylR
Term.
Term.
P u
RBS
phz genes
Term.
Term.
PYOCYANIN

Wetlab - Drylab

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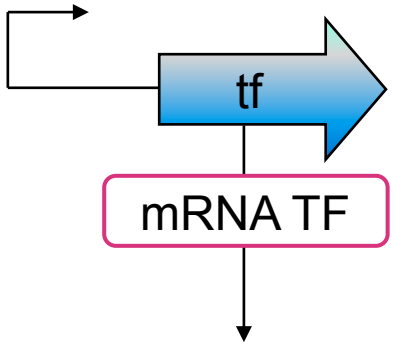
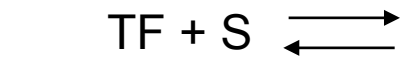
Computational Modelling of the Biosensor

- 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 the hypothesis proposed by the biologists

The Model



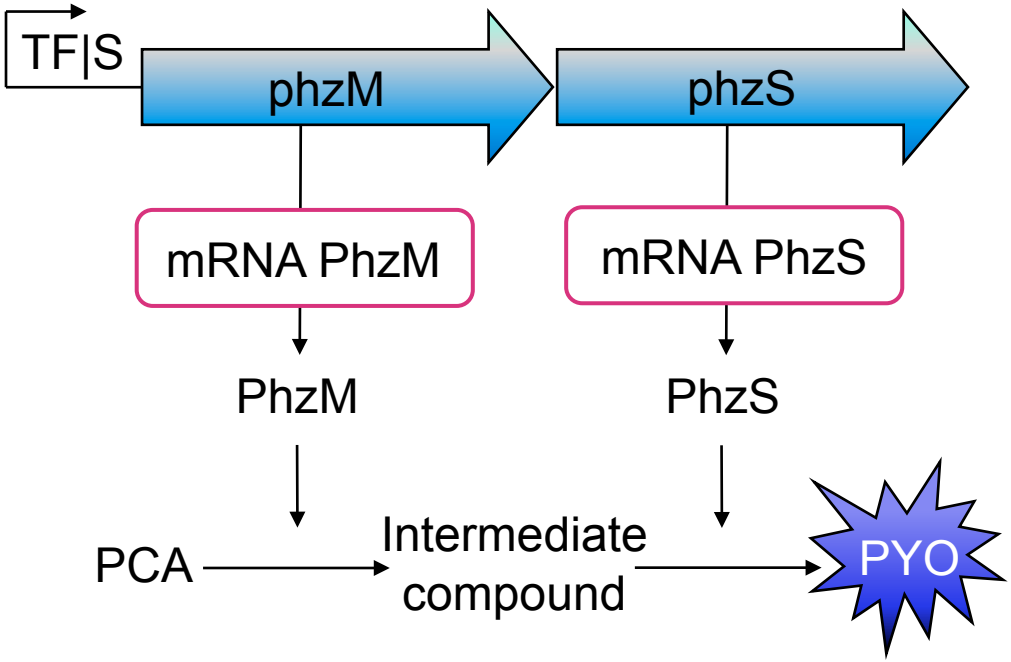
- Merge transcription and translation
- Merge phzM with phzS (Parsons 2007)



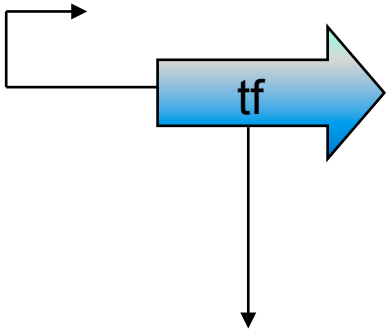
TF: Dntr or Xylr

S: signal

TF|S: complex



The Model



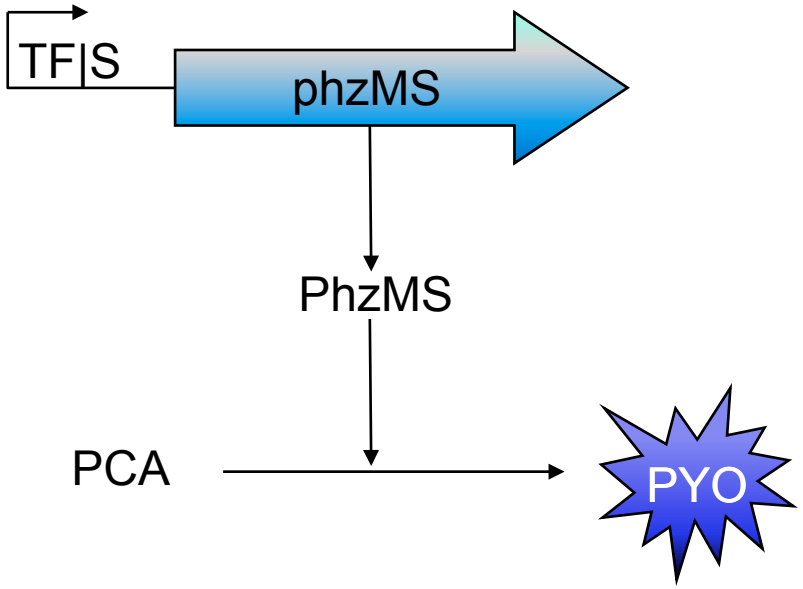
- Merge transcription and translation
- Merge phzM with phzS (Parsons 2007)

TF + S ⇌ TF|S

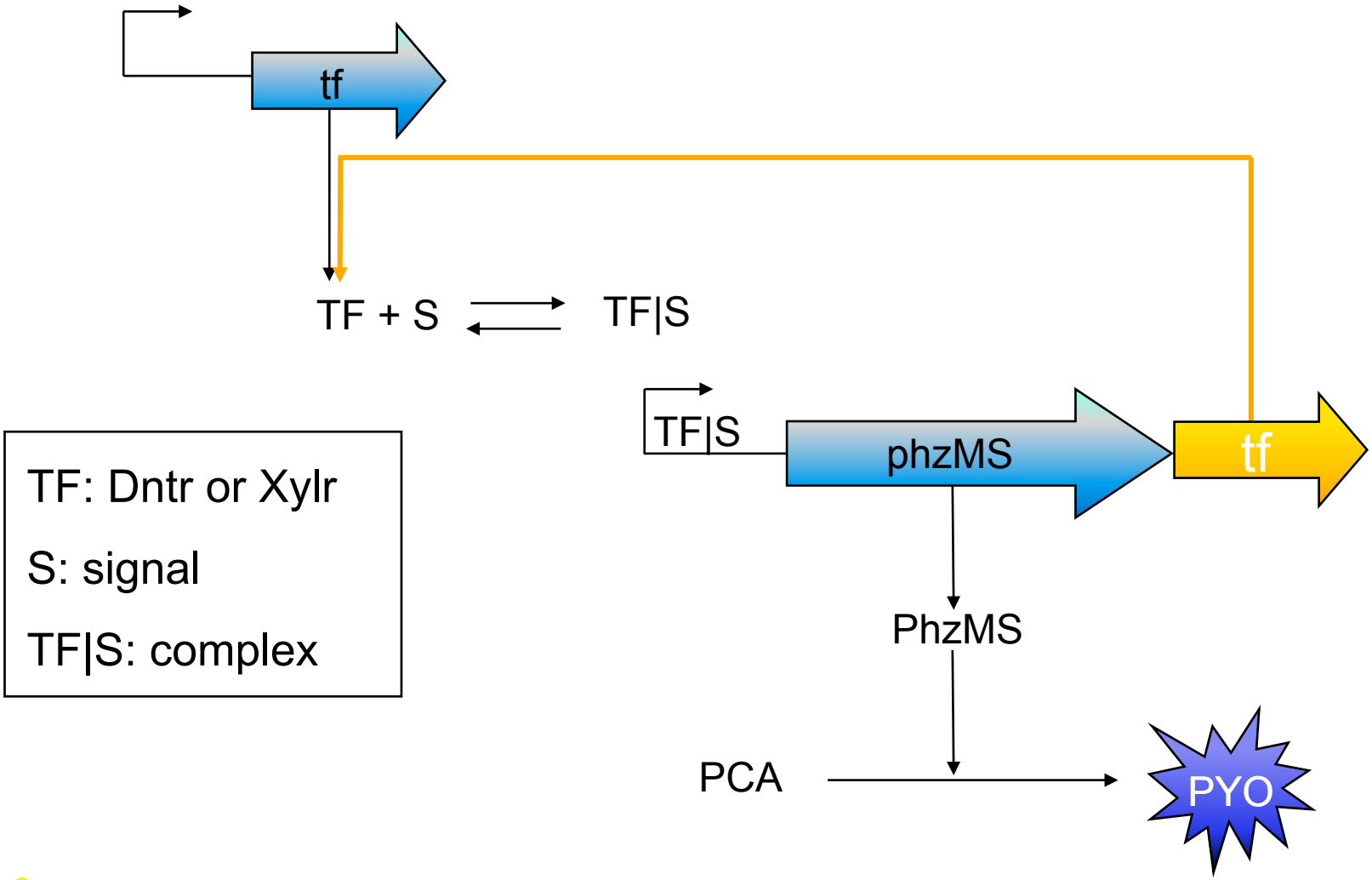
TF: Dntr or Xylr

S: signal

TF|S: complex



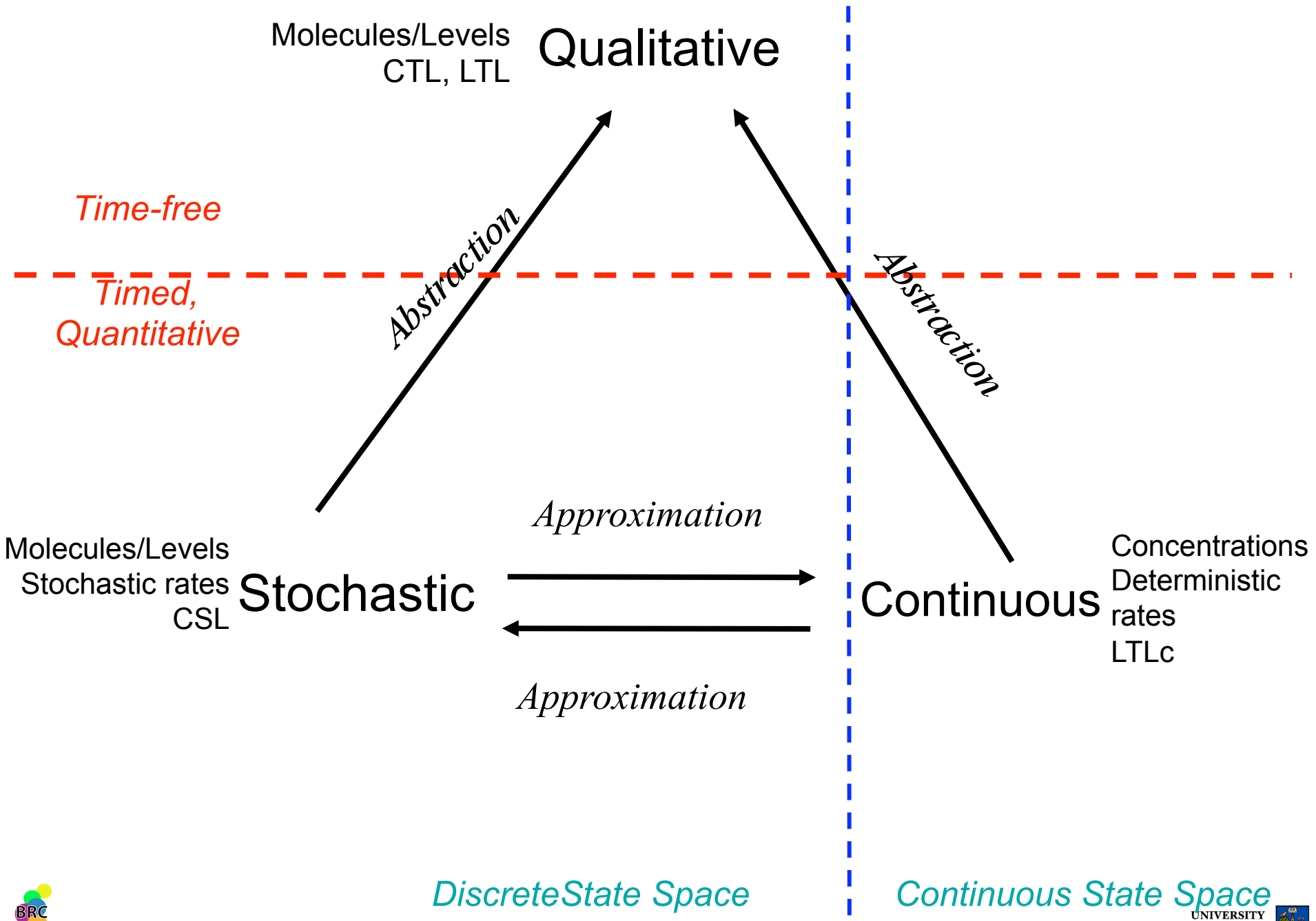
Feedback Loop



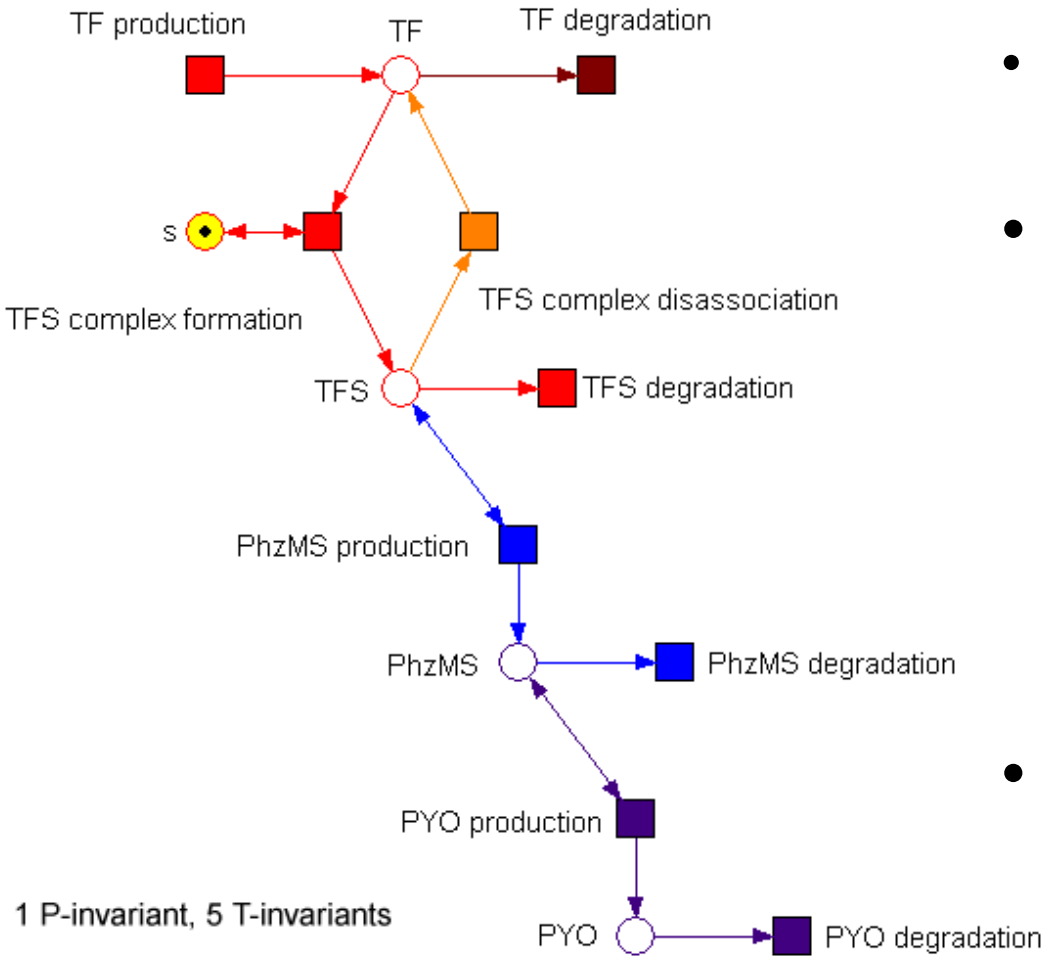
TF: Dntr or Xylr

S: signal

TF|S: complex


Molecules/Levels
CTL, LTL
Qualitative
Time-free
Timed,
Quantitative
Abstraction
Abstraction
Approximation
Molecules/Levels
Stochastic rates
CSL
Stochastic
Continuous
Concentrations
Deterministic
rates
LTLc
Approximation
DiscreteState Space
Continuous State Space

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

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Ordinary Differential Equations

SBML Available!

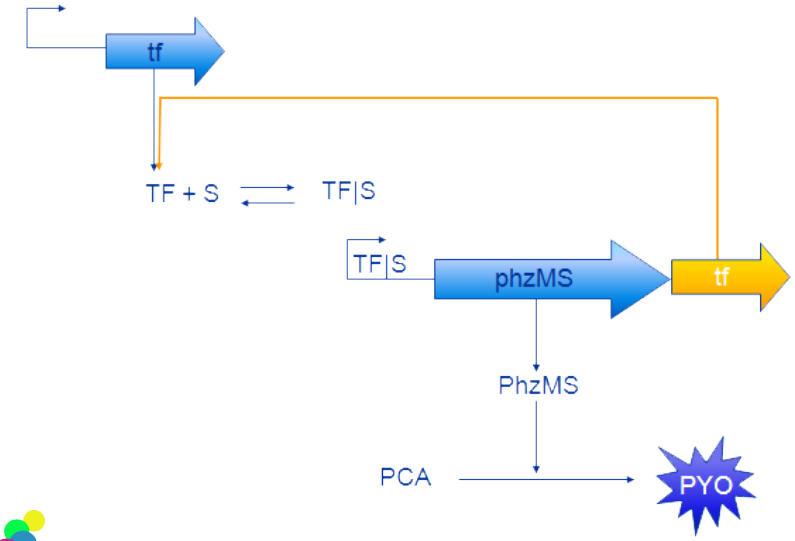
Created in BioNessie

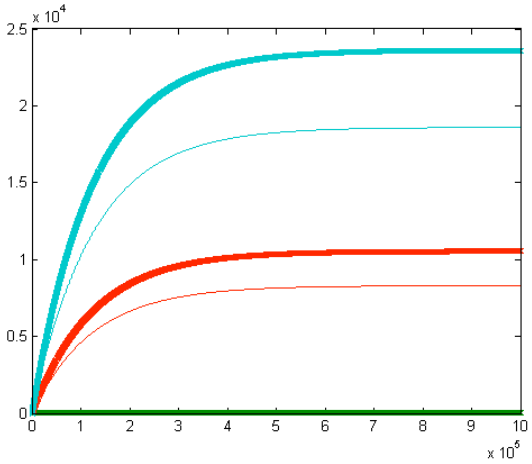
$$\dot{TF} = \alpha_{TF} - \delta_{TF}TF - \beta_{TFS}sTF + k_dTFS + \beta_{TF}\frac{TFS}{\gamma_{TF} + TFS} \quad (1)$$

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

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

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





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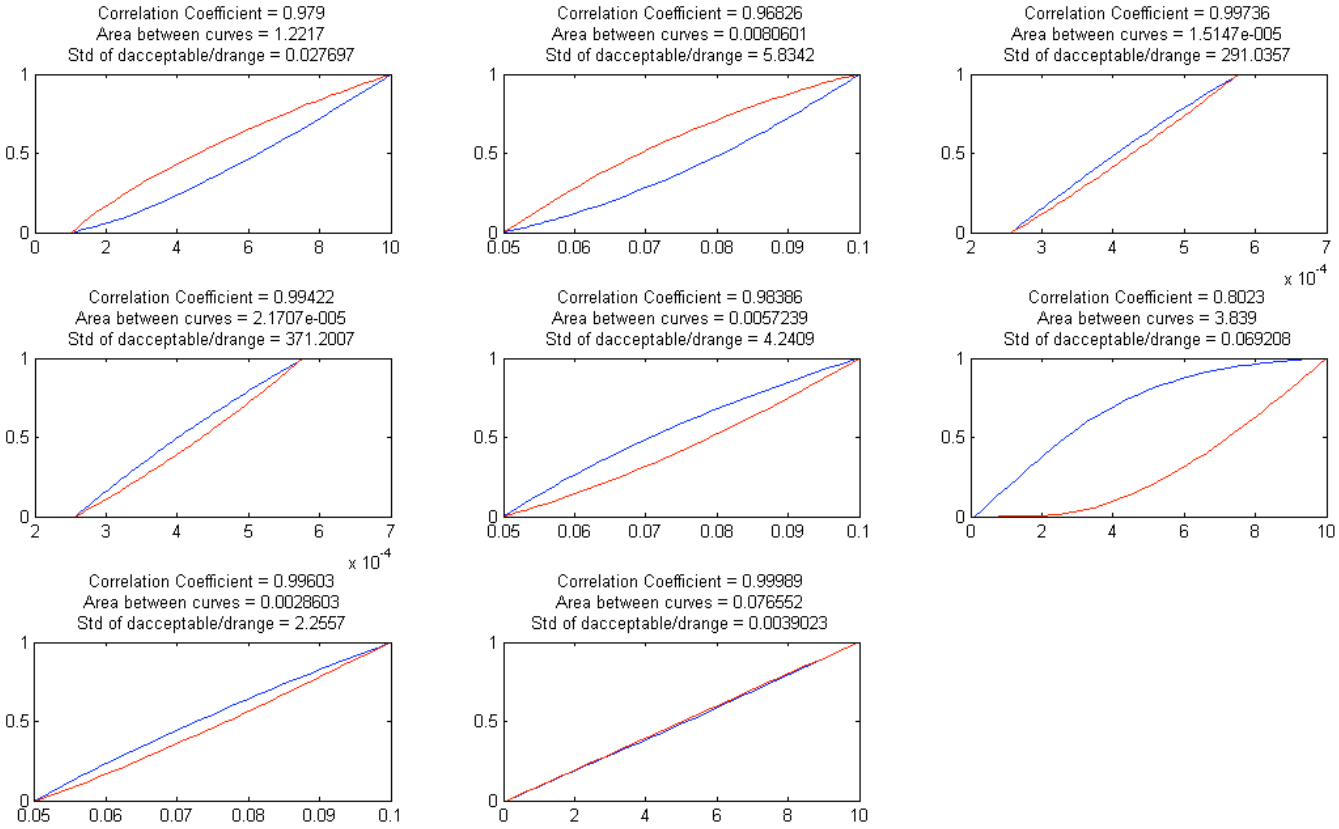
Parameters

- 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

Model Parameter Refinement

- Modified MPSA



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

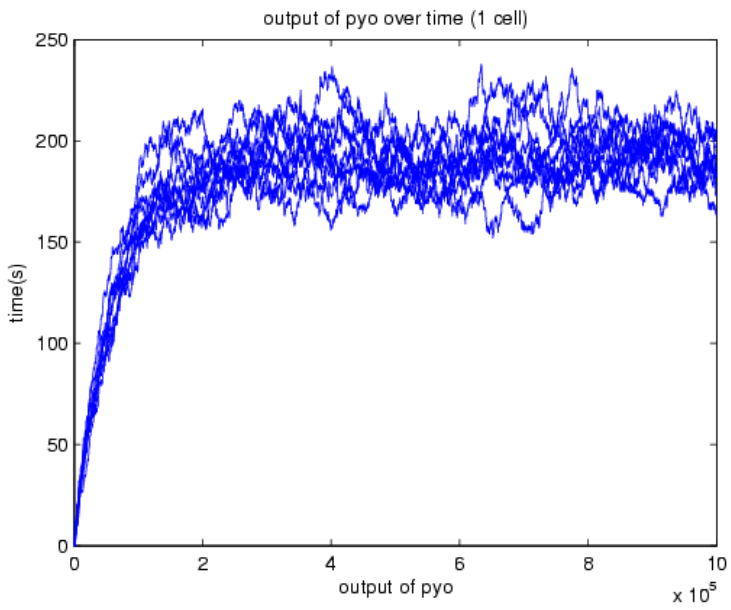
Chemical Master Equations

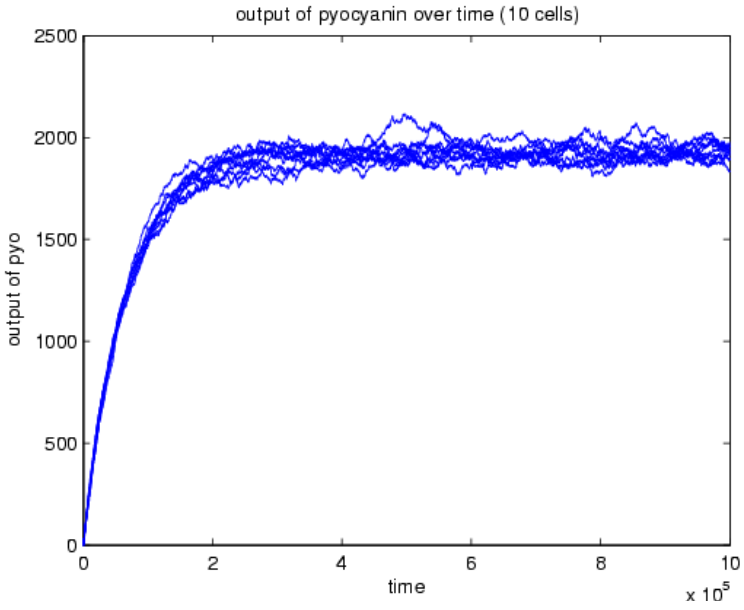
A set of linear, autonomous ODE's, one ODE for each possible state of the system. The system may be written:

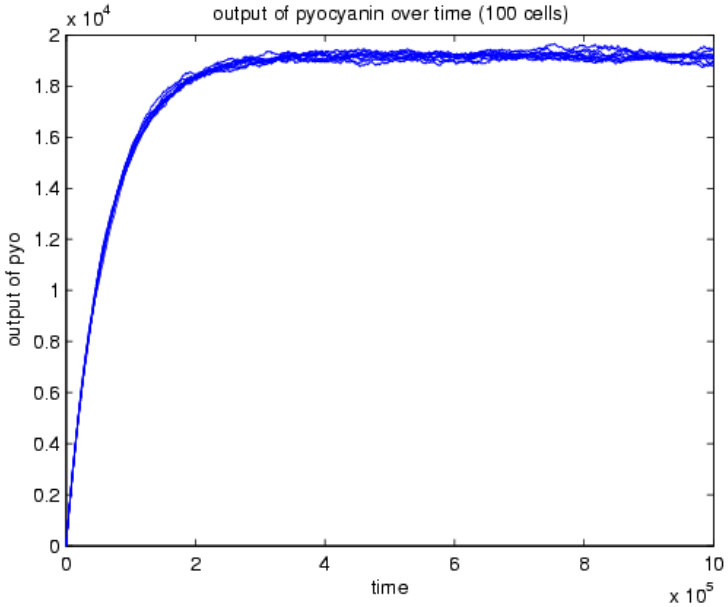
- **Φ → TF** - production of TF
- **TF → Φ** - degradation of TF
- **TF+S → TFS** - association of TFS
- **TFS → TF+S** - dissociation of TFS
- **TFS → Φ** - degradation of TFS
- **Φ → PhzMS** - production of PhzMS
- **PhzMS → Φ** - degradation of PhzMS
- **PhzMS → PYO** - production of pyocyanin
- **PYO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

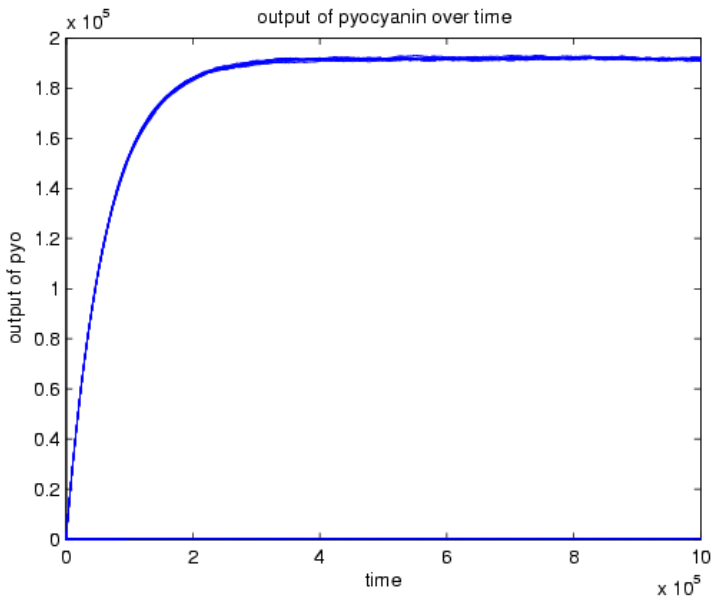
Propensity Functions

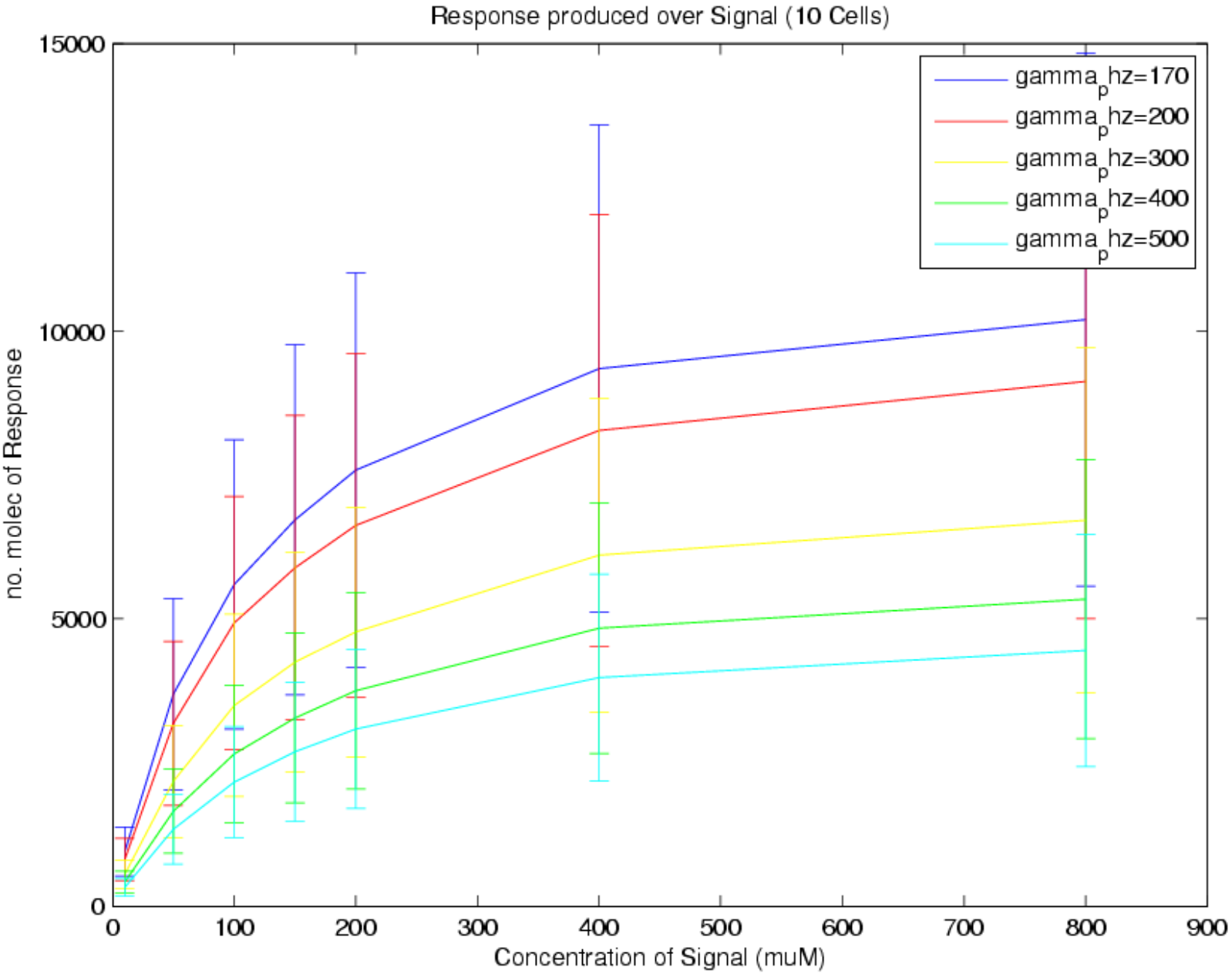
reaction	rate constant	propensity function
$\phi \rightarrow TF$	$\alpha = c(1)$	$a(1) = c(1)$
$TF \rightarrow \phi$	$\delta_{TF} = c(2)$	$a(2) = c(2) * X(1)$
$TF + S \rightarrow TFS$	$K1 * S = c(3)$	$a(3) = c(3) * X(1)$
$TFS \rightarrow TF + S$	$Km1 = c(4)$	$a(4) = c(4) * X(2)$
$TFS \rightarrow \phi$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



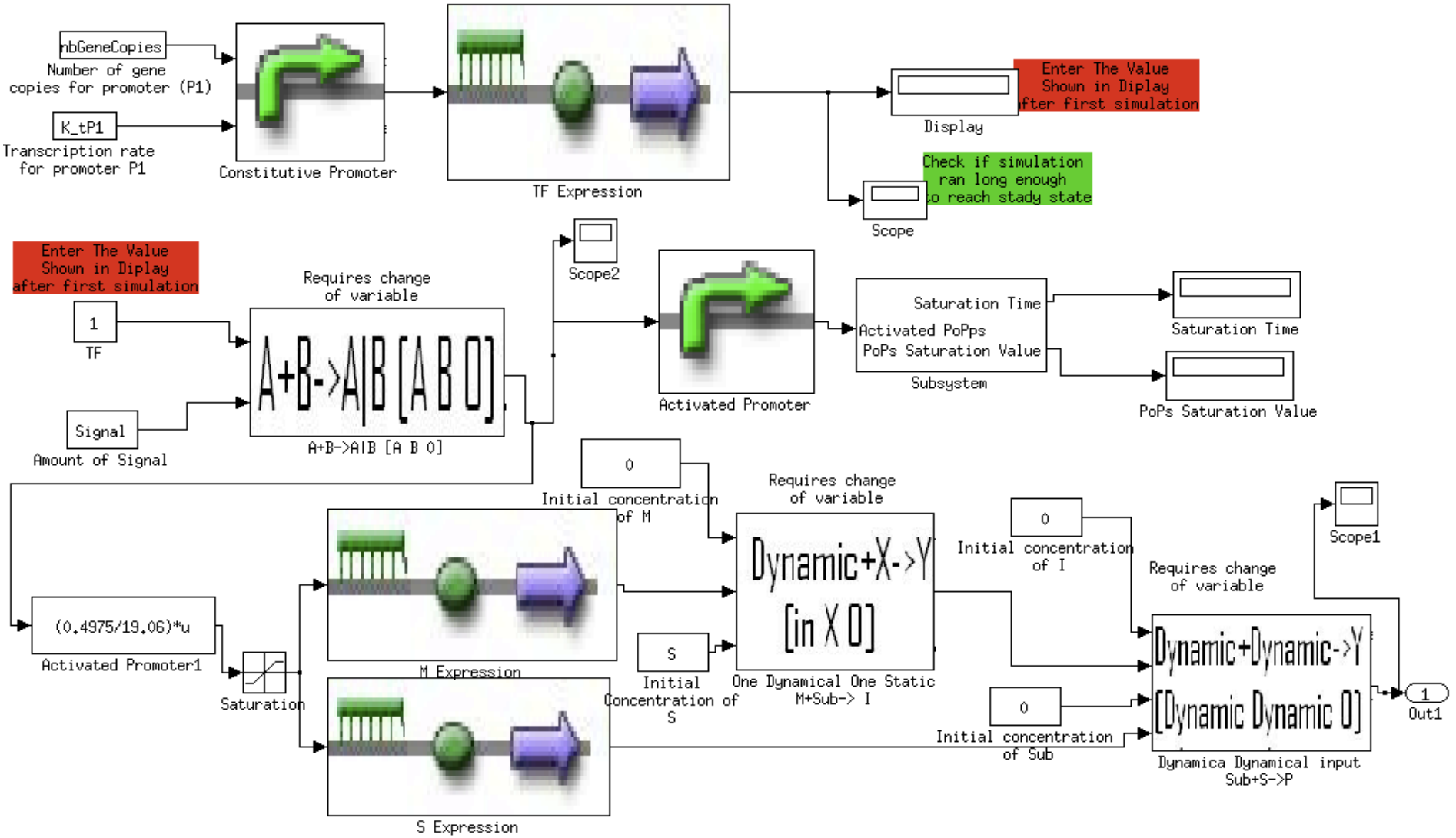








Simulink Modelling Environment



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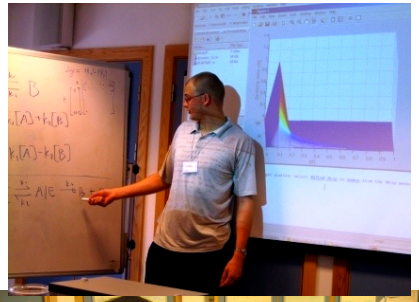
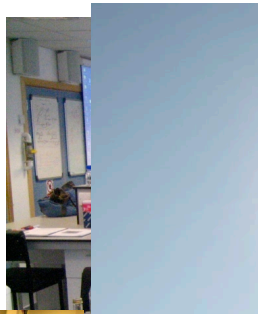
In the end…

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

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





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iGEM team

Students

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

Funders:
Scottish Enterprise
European Union
University of Glasgow

Instructors

- David Forehand
- David Gilbert
- Gary Gray
- Xu Gu
- Raya Khanin
- David Leader
- Susan Rosser
- Emma Travis
- Gabriela Kalna (strathclyde)
- Monika Heiner (cottbus)

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University of Glasgow, UK **www.brc.dcs.gla.ac.uk**

Joan Grindlay **Beatson Institute, Glasgow, UK**

Model checking: Monika Heiner, Sebastian Lehrack (Cottbus)
Robin Donaldson (Glasgow)

CANCER RESEARCH UK

dti

Department of Trade & Industry
Beacon Projects