Modelling the kinetic behaviour of the MAPK cascade: A case study

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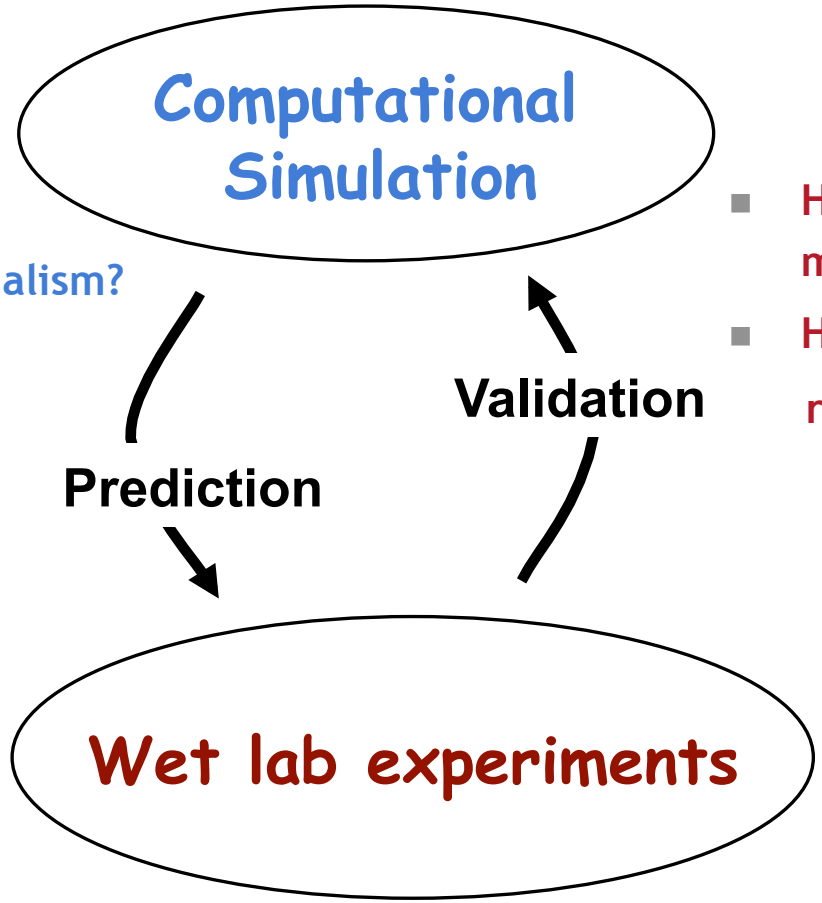
Overview

- Synthetic Biology should be undertaken within the framework of a sound engineering approach
- Systems Biology will play a major role in providing such a framework.
  - To what extent can Systems Biology be regarded as a rigorous discipline incorporating sound analytical principles?
  - Explore how analytical methods from other disciplines can be adapted to the context of Systems Biology.
- Illustration: MAPK (ERK) signalling pathway SysBio project
  - 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 (BPS)- project:

- 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

- Stochastic process algebras
- Petri nets
- Ordinary Differential Equations

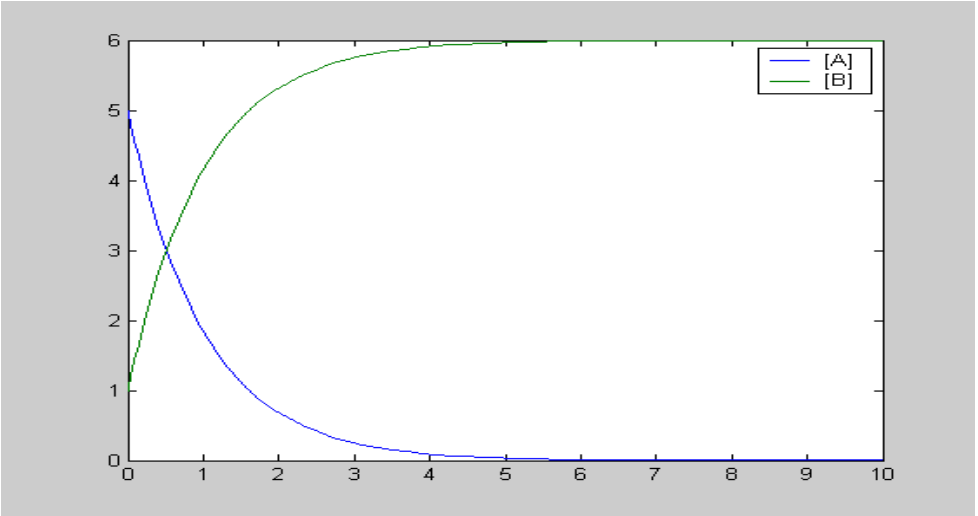
Kinetics and differential equations

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

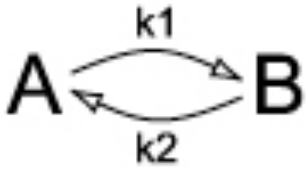
Rate constant = k, with indices indicating constants for various reactions (k_1, k_2...)

$$A \rightarrow B$$

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



Reversible, Single-Molecule Reaction

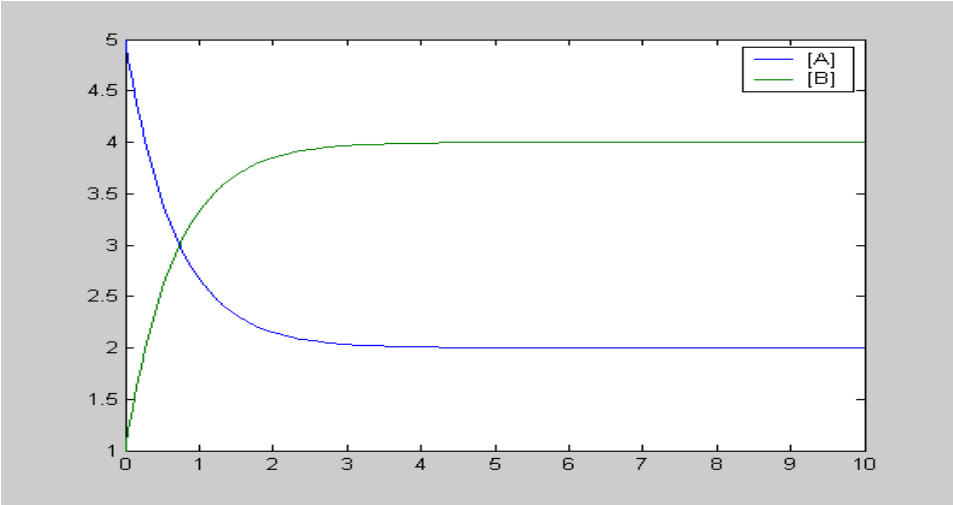


forward reverse

$$\frac{d[A]}{dt} = -k_1[A] + k_2[B]$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B]$$

Rainer Breitling



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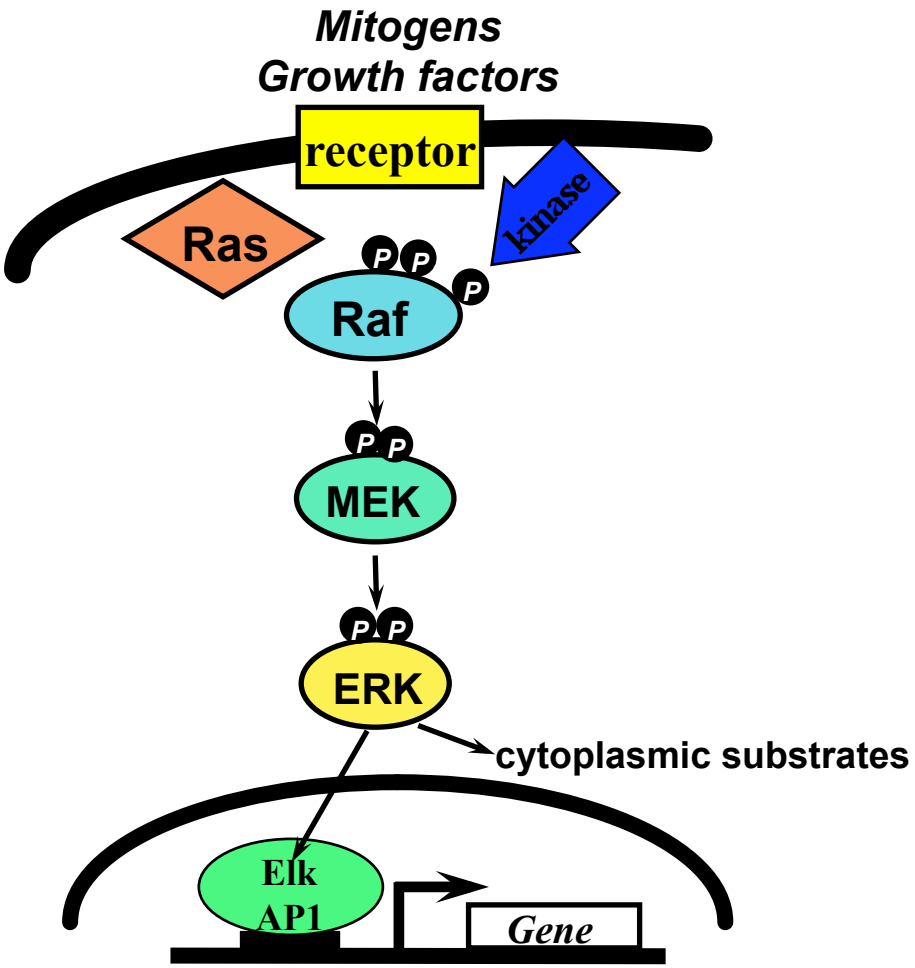
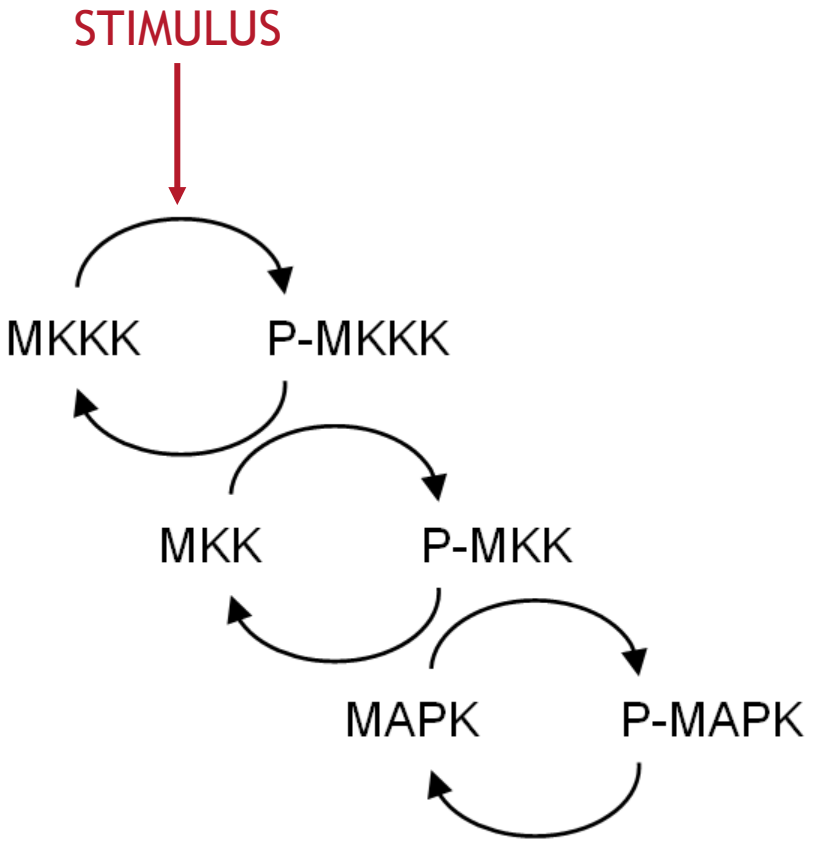
The Ras – Raf – MEK – ERK Signalling pathway

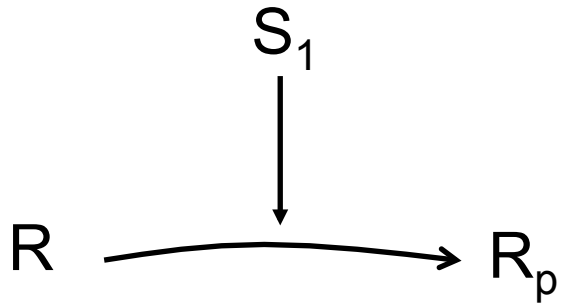


MAPK Pathway

- 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

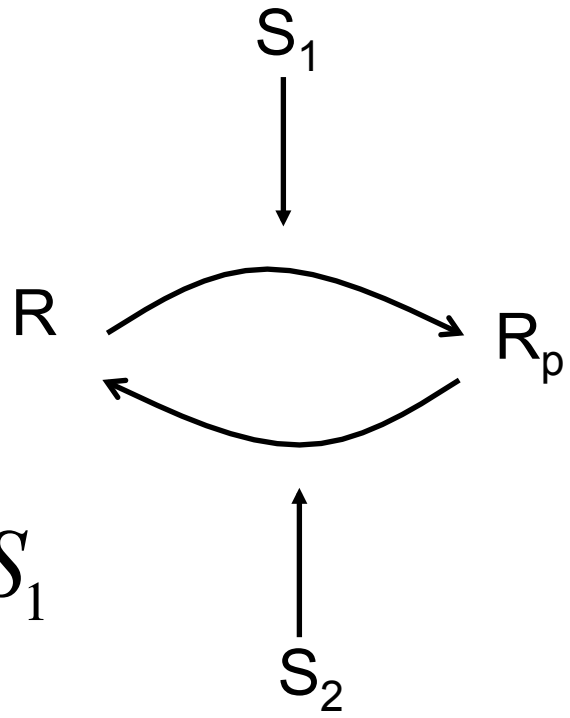


- R: substrate,
- R_p: product (phosphorylated R)
- S_1: enzyme (kinase)
- $R|S_1$ substrate-enzyme complex

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

Phosphorylation - dephosphorylation loop
Mass action model 1

- R: unphosphorylated form
- R_p: phosphorylated form
- S_1: kinase
- S_2: phosphotase
- $R|S_1$ unphosphorylated+kinase complex
- $R|S_2$ unphosphorylated+phosphotase complex



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

$$R+S_2 \xleftarrow{k_3'} R_p|S_2 \underset{k_2'}{\overset{k_1'}{\rightleftarrows}} R_p + S_2$$

Phosphorylation - dephosphorylation loop
Mass action model 1

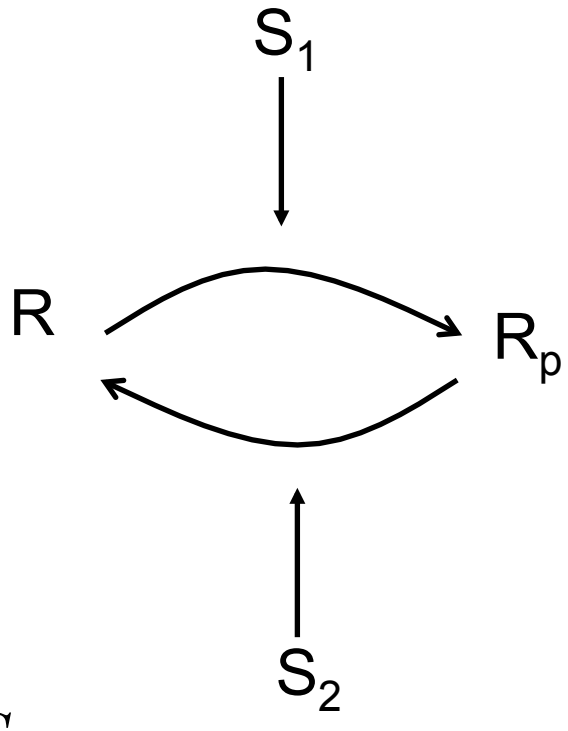
```
k1=100; k2=499; k3=1;
k1dash=100; k2dash=499; k3dash=1;
dydt = [-k1*S1*R + k2*RS1 + k3*RS1                          % S1
        -k1*S1*R + k2*RS1 + k3dash*RpS2                     % R
        +k1*S1*R - k2*RS1 - k3*RS1                          % RS1
        -k1dash*S2*Rp + k2dash*RpS2 + k3*RS1                % Rp
        -k1dash*S2*Rp + k2dash*RpS2 + k3dash*RpS2           % S2
        +k1*Rp*S2 - k2dash*RpS2 - k3dash*RpS2 ];            % RpS2
```

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

$$R + S_2 \xleftarrow{k_3'} R_p \mid S_2 \underset{k_2'}{\overset{k_1'}{\rightleftarrows}} R_p + S_2$$

Phosphorylation - dephosphorylation loop
Mass action model 3 (all singing/dancing)

- R: unphosphorylated form
- R_p: phosphorylated form
- S_1: kinase
- S_2: phosphotase
- $R|S_1$ unphosphorylated+kinase complex
- $R_p|S_1$ phosphorylated+kinase complex
- $R|S_2$ unphosphorylated+phosphotase complex
- $R_p|S_2$ phosphorylated+phosphotase complex



$$R+S_1 \underset{k_2}{\overset{k_1}{\rightleftarrows}} R|S_1 \underset{k_6}{\overset{k_3}{\rightleftarrows}} R_p|S_1 \underset{k5}{\overset{k_4}{\rightleftarrows}} R_p+S_1$$

$$R+S_2 \underset{k_5'}{\overset{k_4'}{\rightleftarrows}} R|S_2 \underset{k_6'}{\overset{k_3'}{\rightleftarrows}} R_p|S_2 \underset{k_2'}{\overset{k_1'}{\rightleftarrows}} R_p+S_2$$

Michaelis-Menten equation

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E \mid S \xrightarrow{k_3} E + P$$

- E: enzyme, S: substrate, E|S: enzyme-substrate complexP: product
- Assumptions:
  1. No product reverts to initial substrate
  2. MM Equation holds at initial stage of reaction before concentration of product is appreciable
  3. [E] << [S]
- K_m is [S] at which the reaction rate is half its maximum value

$$V = V_{\max} \times \frac{[S]}{[S] + K_m}$$

Michaelis-Menten equation for phosphorylation-dephosphorylation

$$\frac{dR_p}{dt} = \frac{k_3 \times S_1 \times R}{K_{m1} + R} - \frac{k_3' \times R_p}{K_{m2} + R_p}$$

- dR_p/dt == reaction rate V
- k_3 x S_1 == V_{max} for the forward reaction
- k_3' == V_{max} for the reverse reaction (S_2 is ignored)
- K_{m1} == $(k_2+k_3)/k_1$ (k's from mass-action 1)

Phosphorylation cascade: 3-stage, Mass-Action model 1

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

$$R + S_2 \xleftarrow{k_3'} R_p \mid S_2 \underset{k_2'}{\overset{k_1'}{\rightleftarrows}} R_p + S_2$$

$$SS_1 = R_p$$

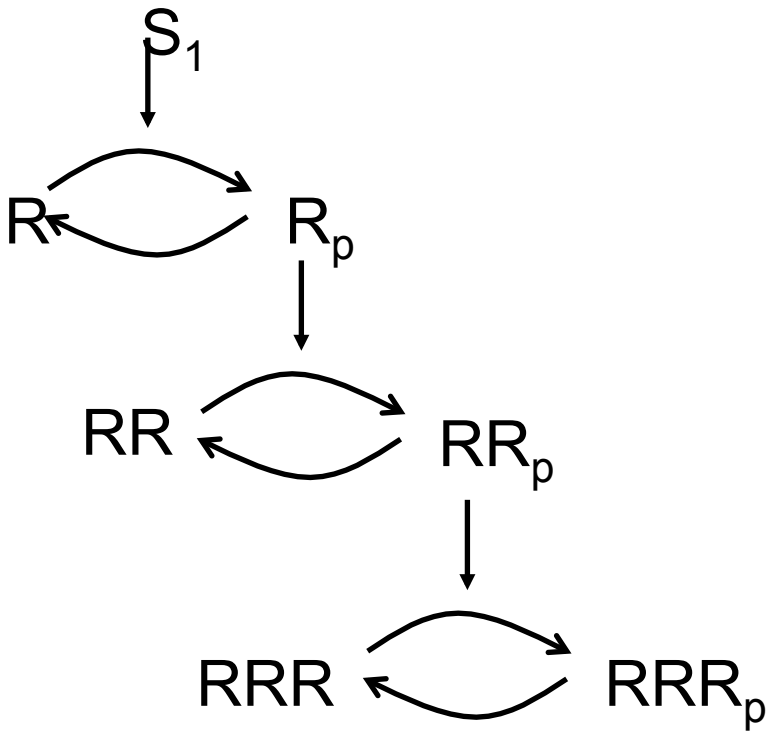
$$RR + SS_1 \underset{kk_2}{\overset{kk_1}{\rightleftarrows}} RR \mid SS_1 \xrightarrow{kk_3} RR_p + SS_1$$

$$RR + SS_2 \xleftarrow{kk_3'} RR_p \mid SS_2 \underset{kk_2'}{\overset{kk_1'}{\rightleftarrows}} RR_p + SS_2$$

$$SSS_1 = RR_p$$

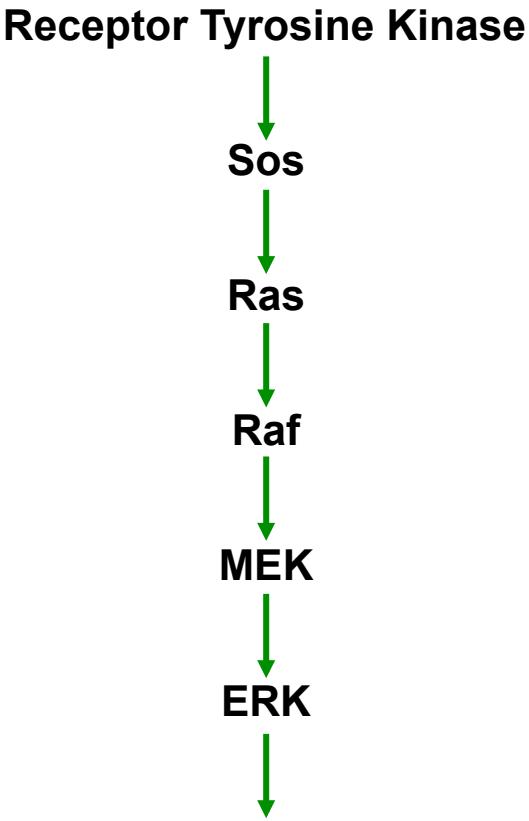
$$RRR + SSS_1 \underset{kkk_2}{\overset{kkk_1}{\rightleftarrows}} RRR \mid SSS_1 \xrightarrow{kkk_3} RRR_p + SSS_1$$

$$RRR + SSS_2 \xleftarrow{kkk_3'} RRR_p \mid SSS_2 \underset{kkk_2'}{\overset{kkk_1'}{\rightleftarrows}} RRR_p + SSS_2$$



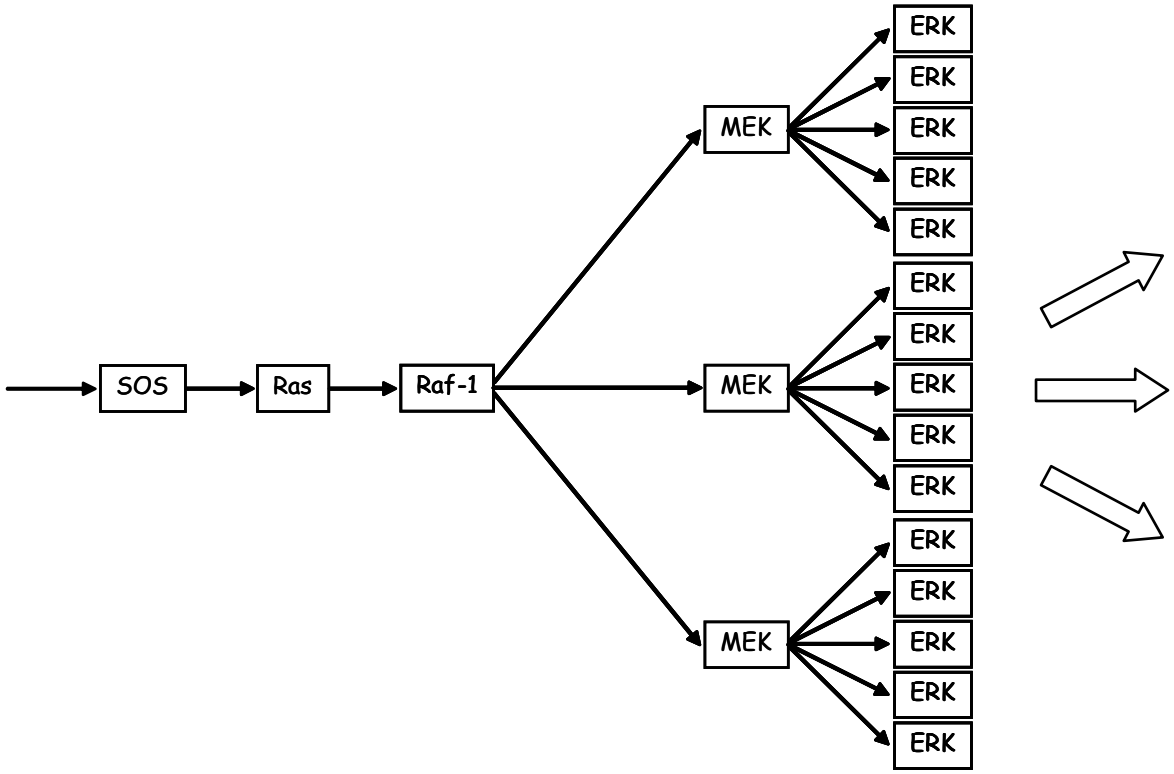
```
dydt = [-k1*S1*R + k2*RS1 + k3*RS1                                          % S1
        -k1*S1*R + k2*RS1 + k3dash*RpS2                                     % R
        +k1*S1*R - k2*RS1 - k3*RS1                                          % RS1
        -k1dash*S2*Rp + k2dash*RpS2 + k3*RS1 - kk1*Rp*RR + kk2*RRSS1 + kk3*RRSS1
                                                                            % Rp = SS1
        -k1dash*S2*Rp + k2dash*RpS2 + k3dash*RpS2                           % S2
        +k1*Rp*S2 - k2dash*RpS2 - k3dash*RpS2                               % RpS2
        -kk1*SS1*RR + kk2*RRSS1 + kk3*RRSS1                                 % SS1 ignored dummy
        -kk1*SS1*RR + kk2*RRSS1 + kk3dash*RRpSS2                            % RR
        +kk1*SS1*RR - kk2*RRSS1 - kk3*RRSS1                                 % RRSS1
        -kk1dash*SS2*RRp + kk2dash*RRpSS2 + kk3*RRSS1 - kkk1*SSS1*RRR + kkk2*RRRSSS1 +
    kkk3*RRRSSS1                                                            % RRp=SSS1
        -kk1dash*SS2*RRp + kk2dash*RRpSS2 + kk3dash*RRpSS2                  % SS2
        +kk1*RRp*SS2 - kk2dash*RRpSS2 - kk3dash*RRpSS2                      % RRpSS2
        -kkk1*SSS1*RRR + kkk2*RRRSSS1 + kkk3*RRRSSS1                        % SSS1 ignored dummy
        -kkk1*SSS1*RRR + kkk2*RRRSSS1 + kkk3dash*RRRpSSS2                   % RRR
        +kkk1*SSS1*RRR - kkk2*RRRSSS1 - kkk3*RRRSSS1                        % RRRSSS1
        -kkk1dash*SSS2*RRRp + kkk2dash*RRRpSSS2 + kkk3*RRRSSS1              % RRRp
        -kkk1dash*SSS2*RRRp + kkk2dash*RRRpSSS2 + kkk3dash*RRRpSSS2         % SSS2
        +kkk1*RRRp*SSS2 - kkk2dash*RRRpSSS2 - kkk3dash*RRRpSSS2 ];          % RRRpSSS2
```

ERK Cascade



Amplification

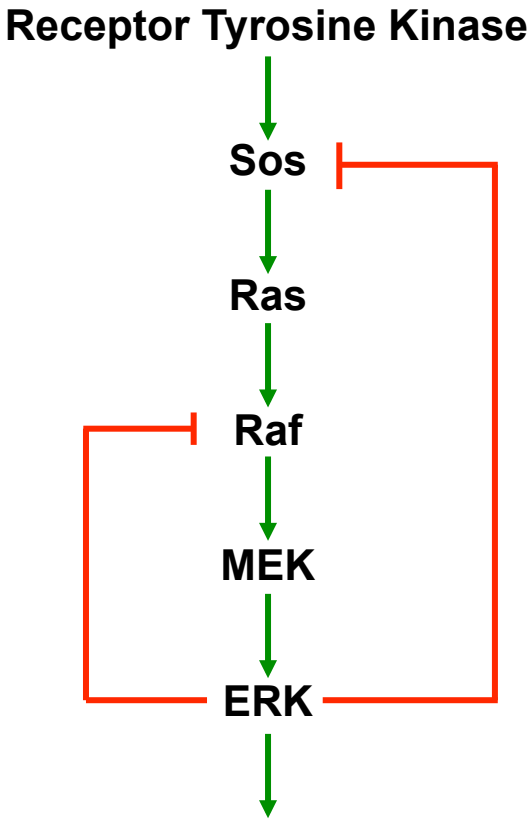
- ERK cascade well known biological amplifier -- amplifies the original signal to create effective cellular responses.
- 1:3:5 are the approximate ratios of Raf-1, MEK and ERK in fibroblasts.



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)



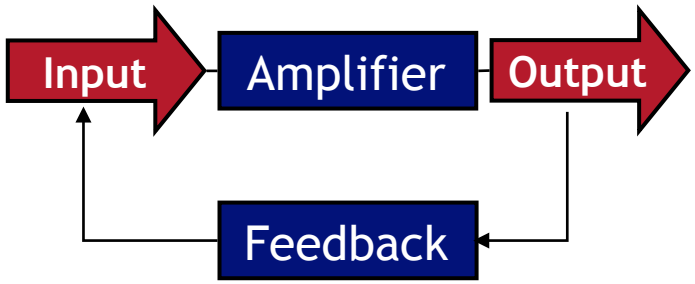
Dougherty *et al*. (2005), Regulation of Raf-1 by Direct Feedback Phosphorylation, Molecular Cell 17 215-224

Is the ERK pathway a negative feedback amplifier?

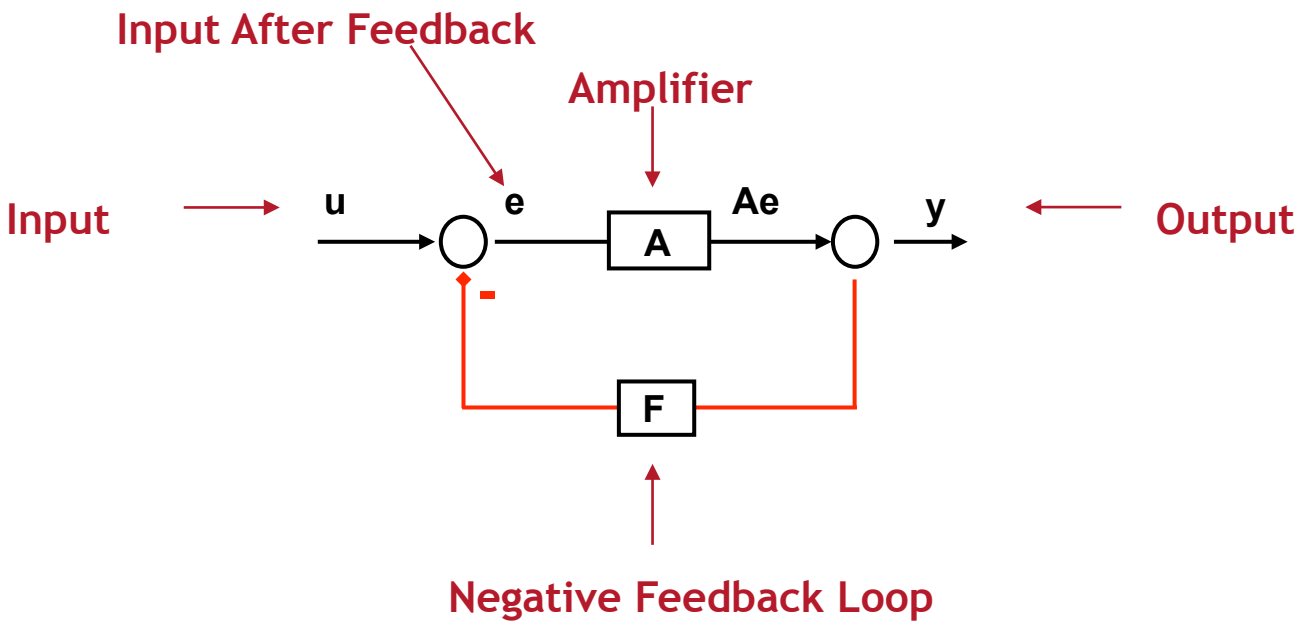
Sauro HM, Kholodenko BN.
Quantitative analysis of signaling networks.
Prog Biophys Mol Biol. 2004 Sep;86(1):5-43.

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



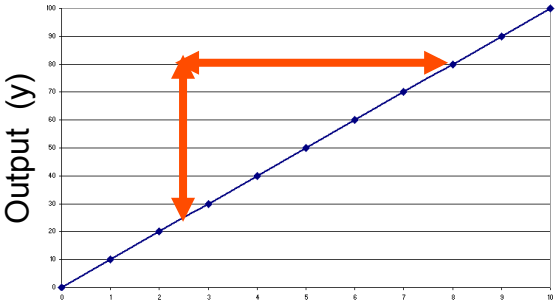
Steady State Equation $$y = \frac{Au}{1 + AF}$$

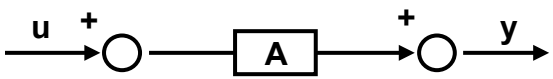
$y = Ae$
$e = u - Fy$

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

The negative feedback imparts signalling robustness

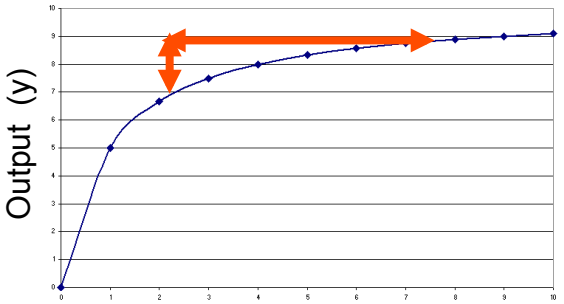
Standard Amplifier

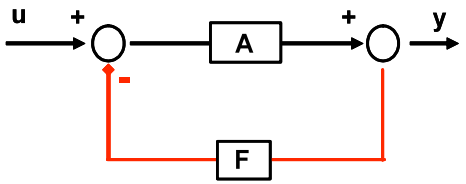




y=A*u

Negative Feedback Amplifier





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

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

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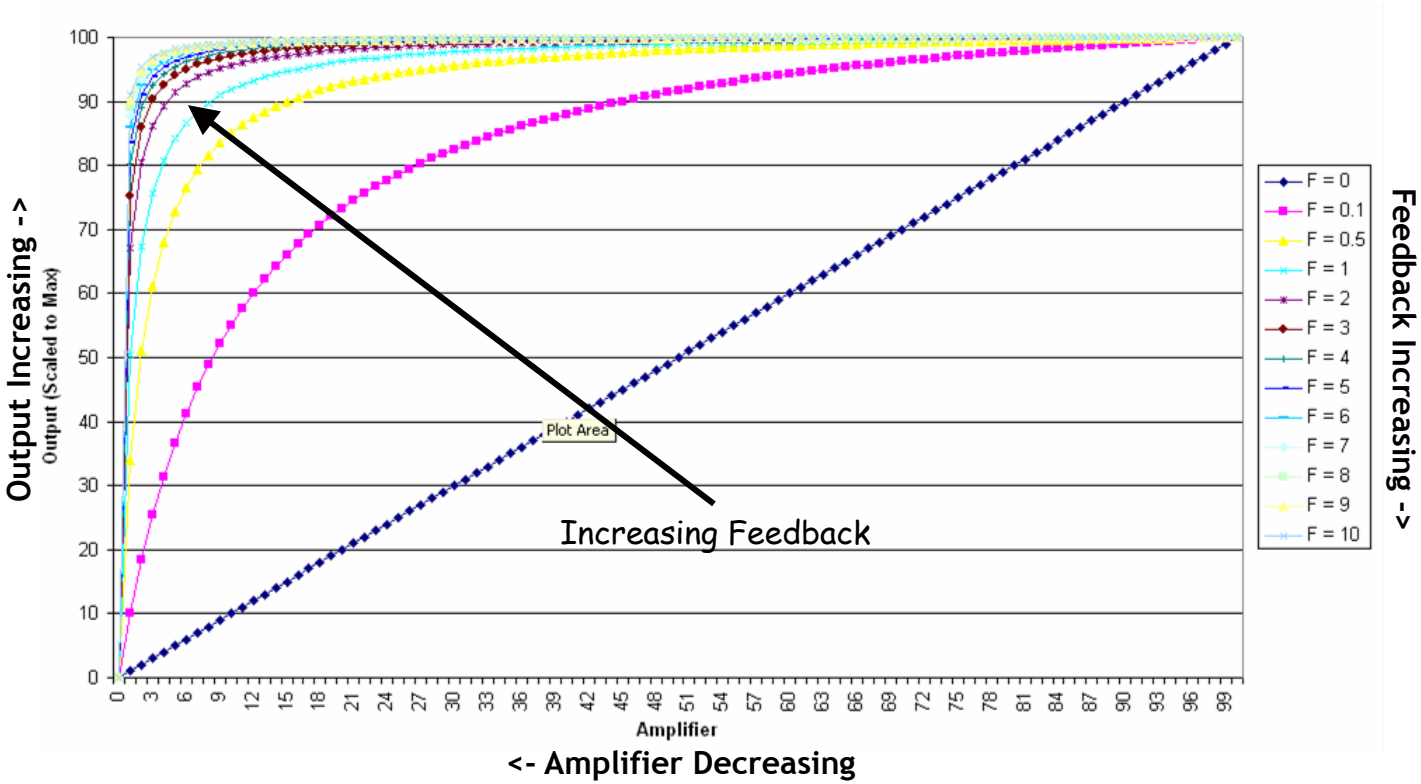
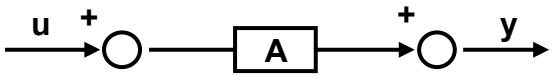
Feedback


Output Increasing ->
Output (Scaled to Max)
Plot Area
Increasing Feedback
Amplifier
<- Amplifier Decreasing
Feedback Increasing ->
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

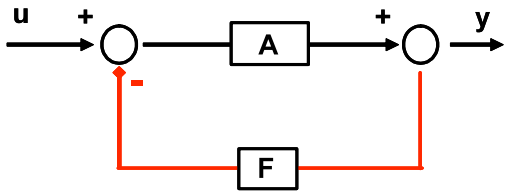
The negative feedback imparts signalling robustness

Standard Amplifier



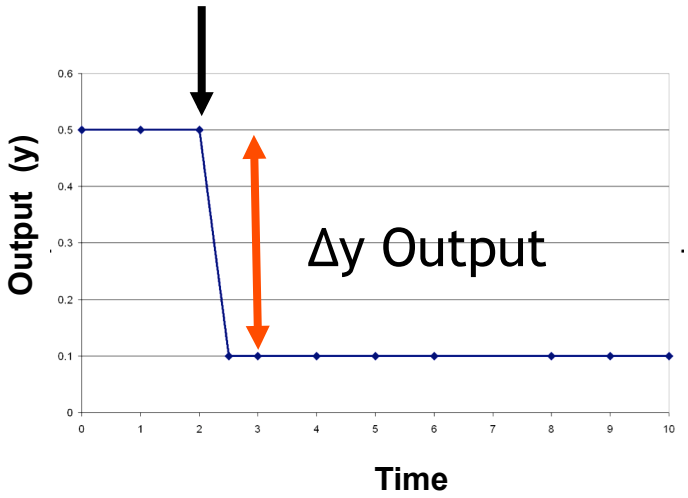
y=A*u

Negative Feedback Amplifier

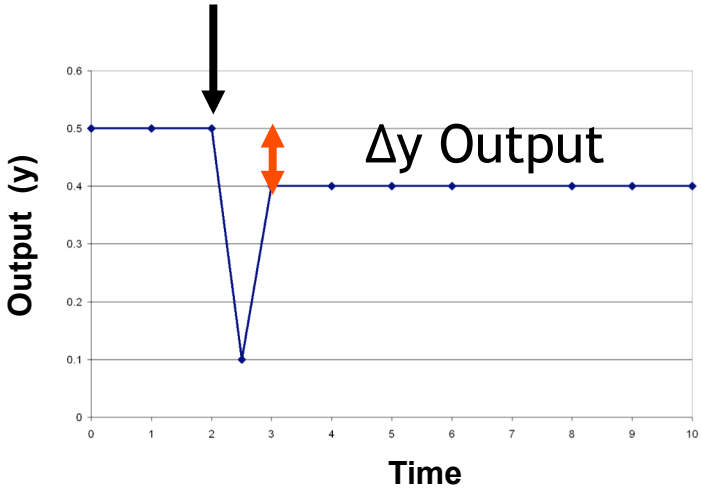


y=A*u/(1+A*F)

Sudden drop in Amplifier (A) gain

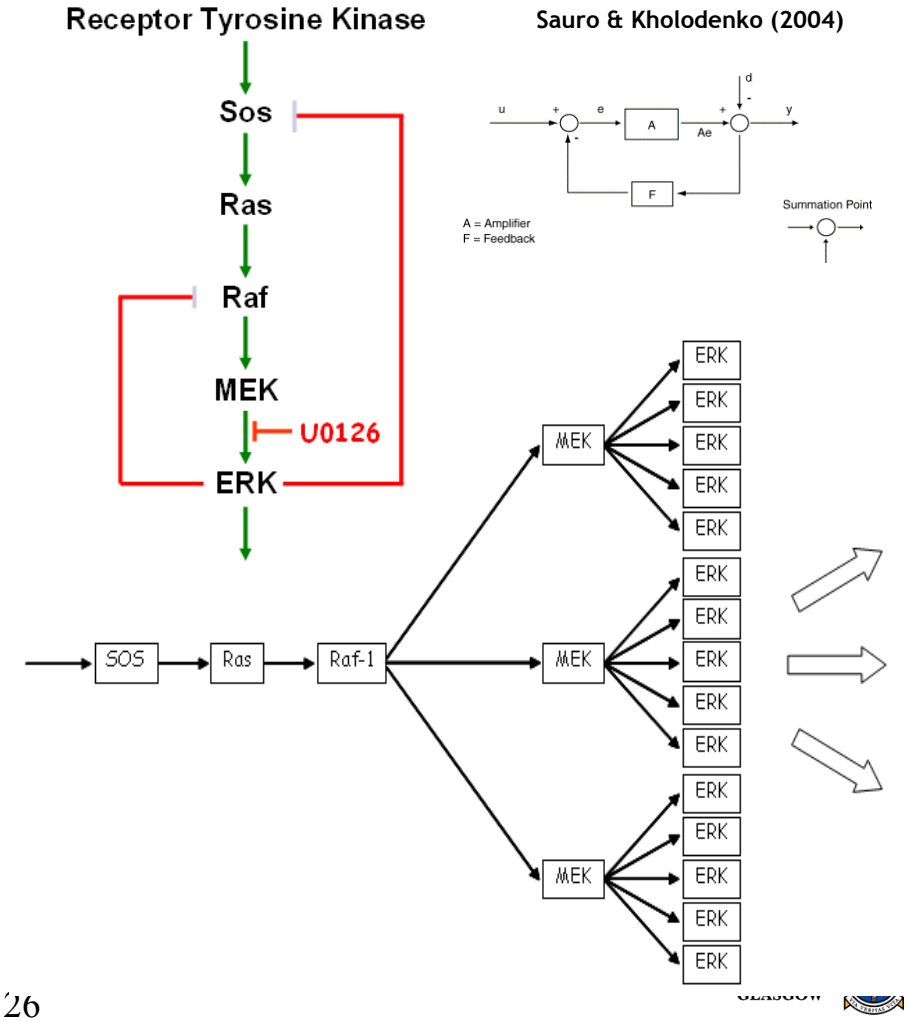


Sudden drop in Amplifier (A) gain

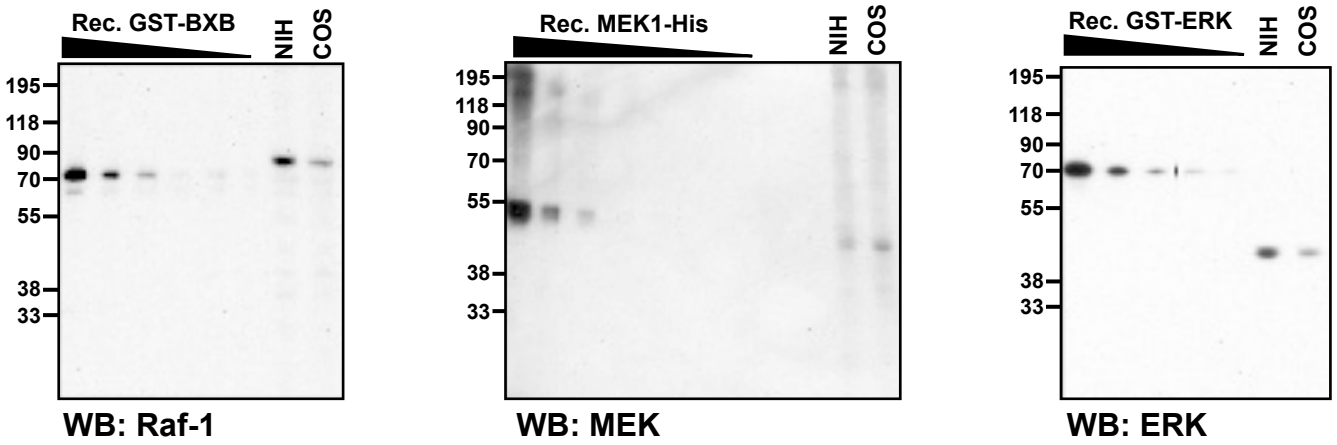


Application to Biology

- 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.



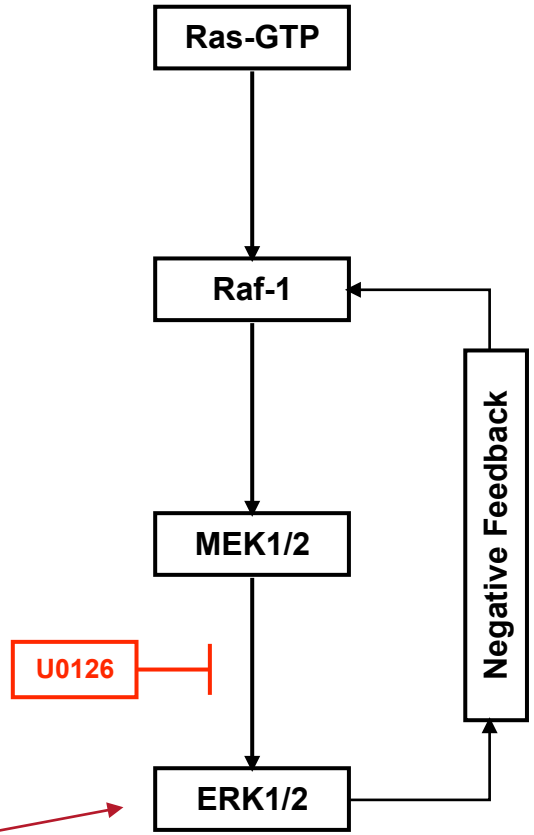
Raf/MEK/ERK amplifies the signal



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 can we test if the ERK pathway is a NFA?

Generate input:
Stimulate with GF



Remove negative feedback

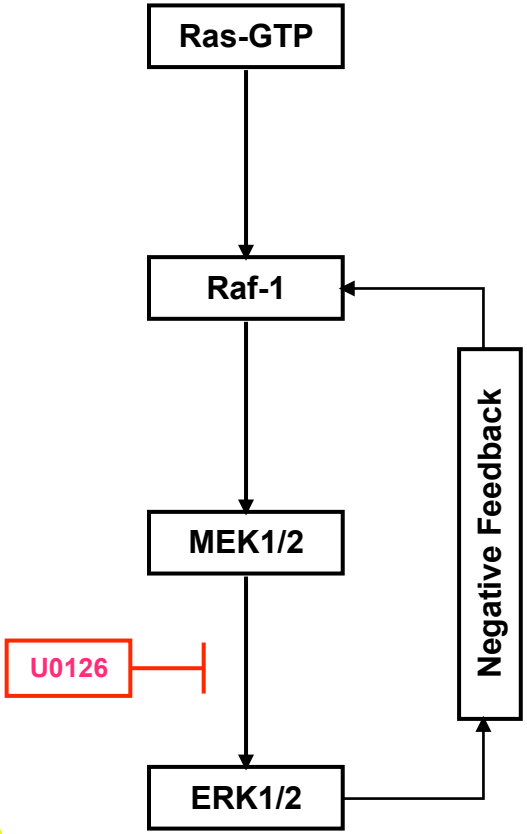
"Disturb the Amplifier":
Use a MEK inhibitior, such as U0126

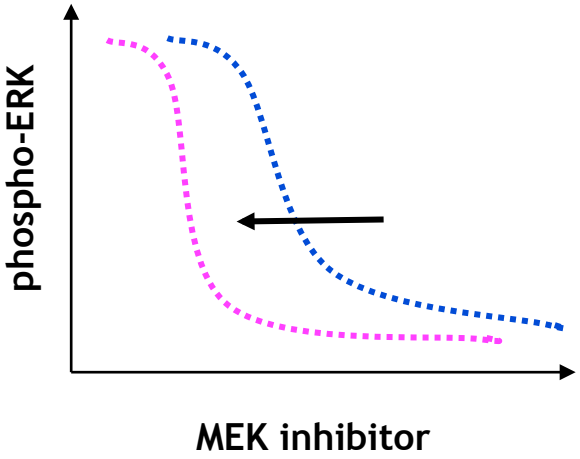
U0126

Measure signal output:
i.e. ERK phosphorylation

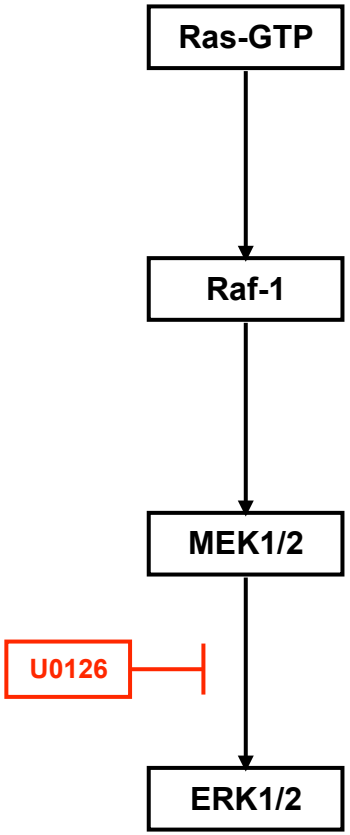
Hypothesis: Braking the feedback should sensitise the ERK pathway to MEK-inhibitor

Feedback intact





Feedback removed



How can we test if the ERK pathway is a NFA?

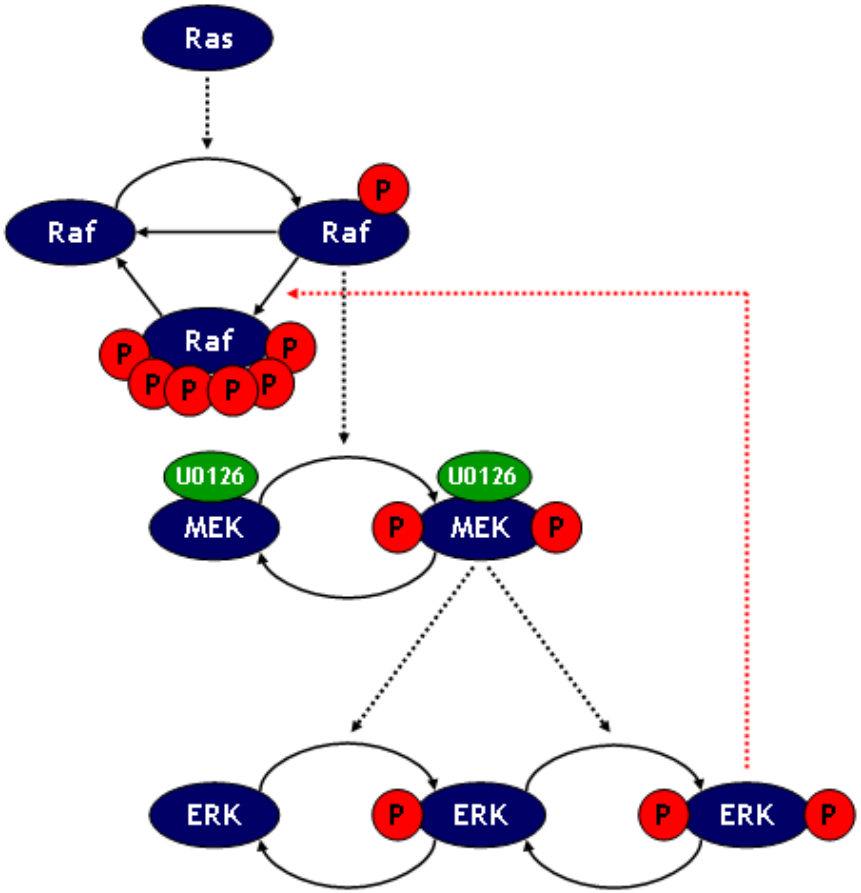
Strategy

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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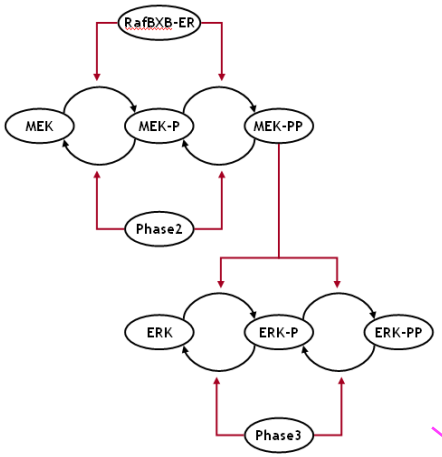
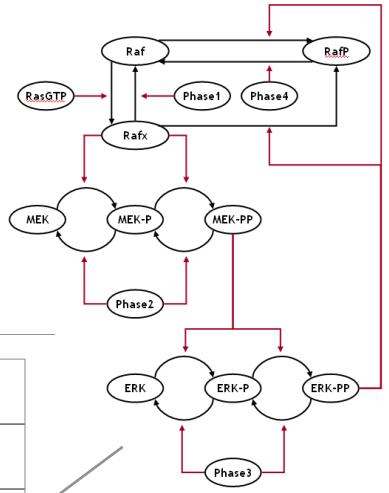
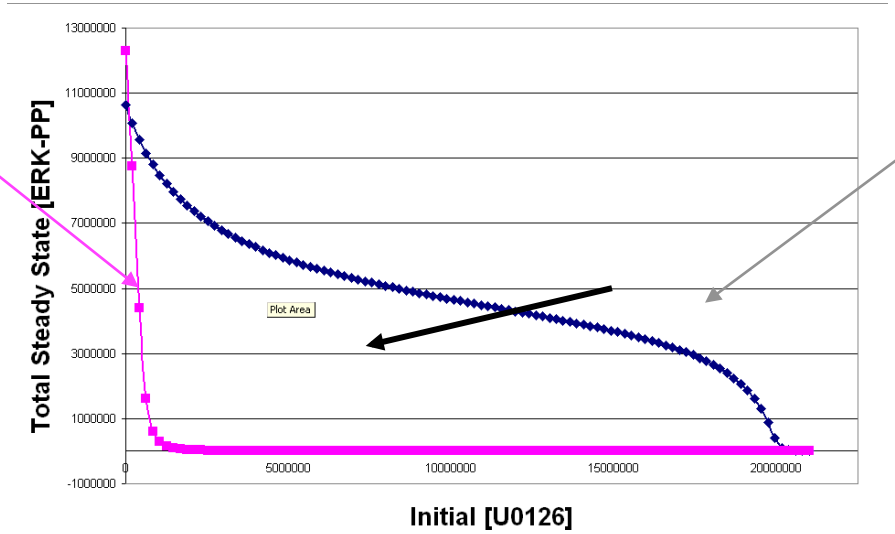
Computational Modeling 2: Results

Feedback broken



Feedback intact

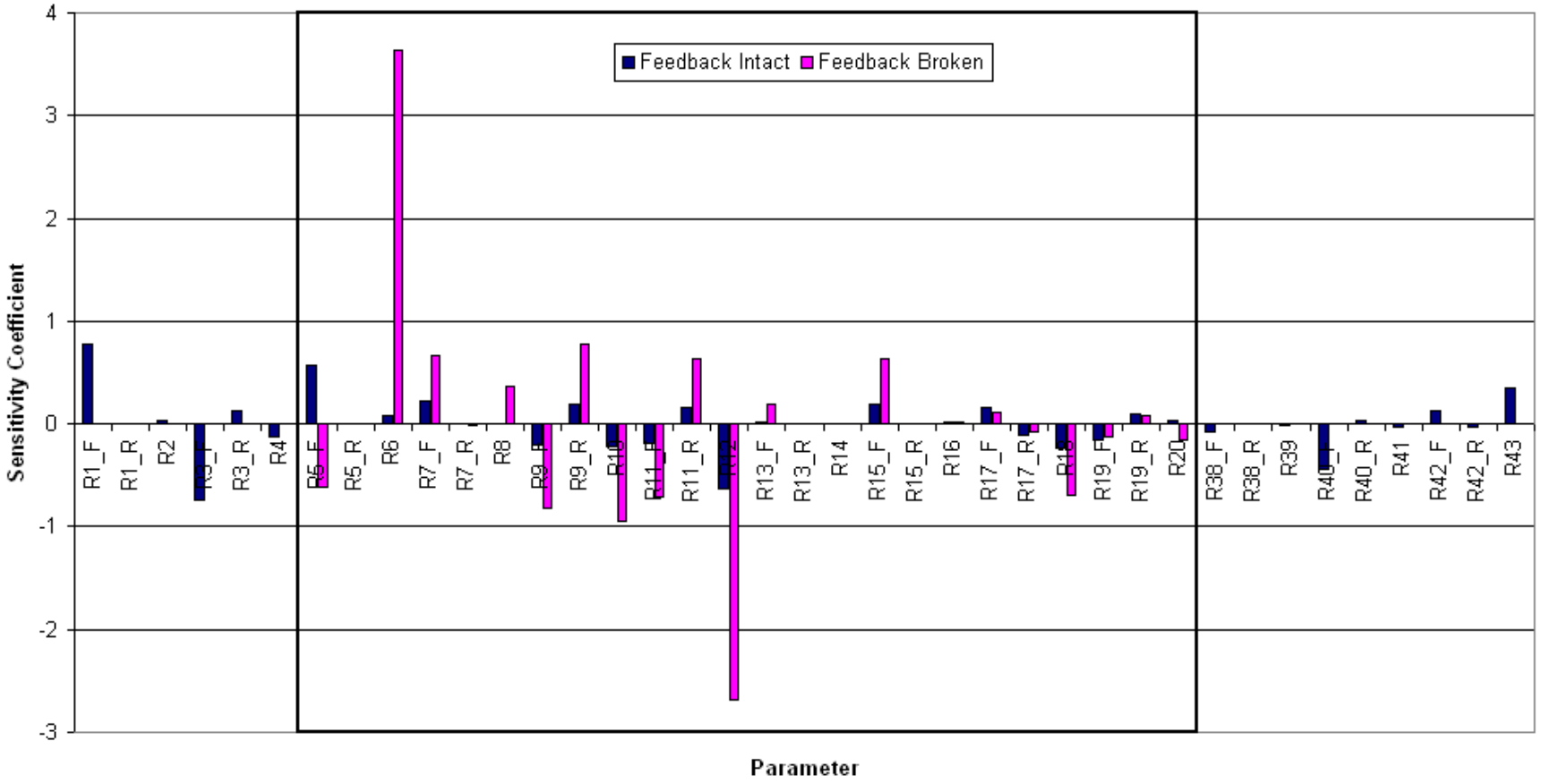




Prediction: Braking the feedback modulates drug response

Computational Modeling 2: Results

Sensitivity of kinetic parameters is decreased due to Negative Feedback



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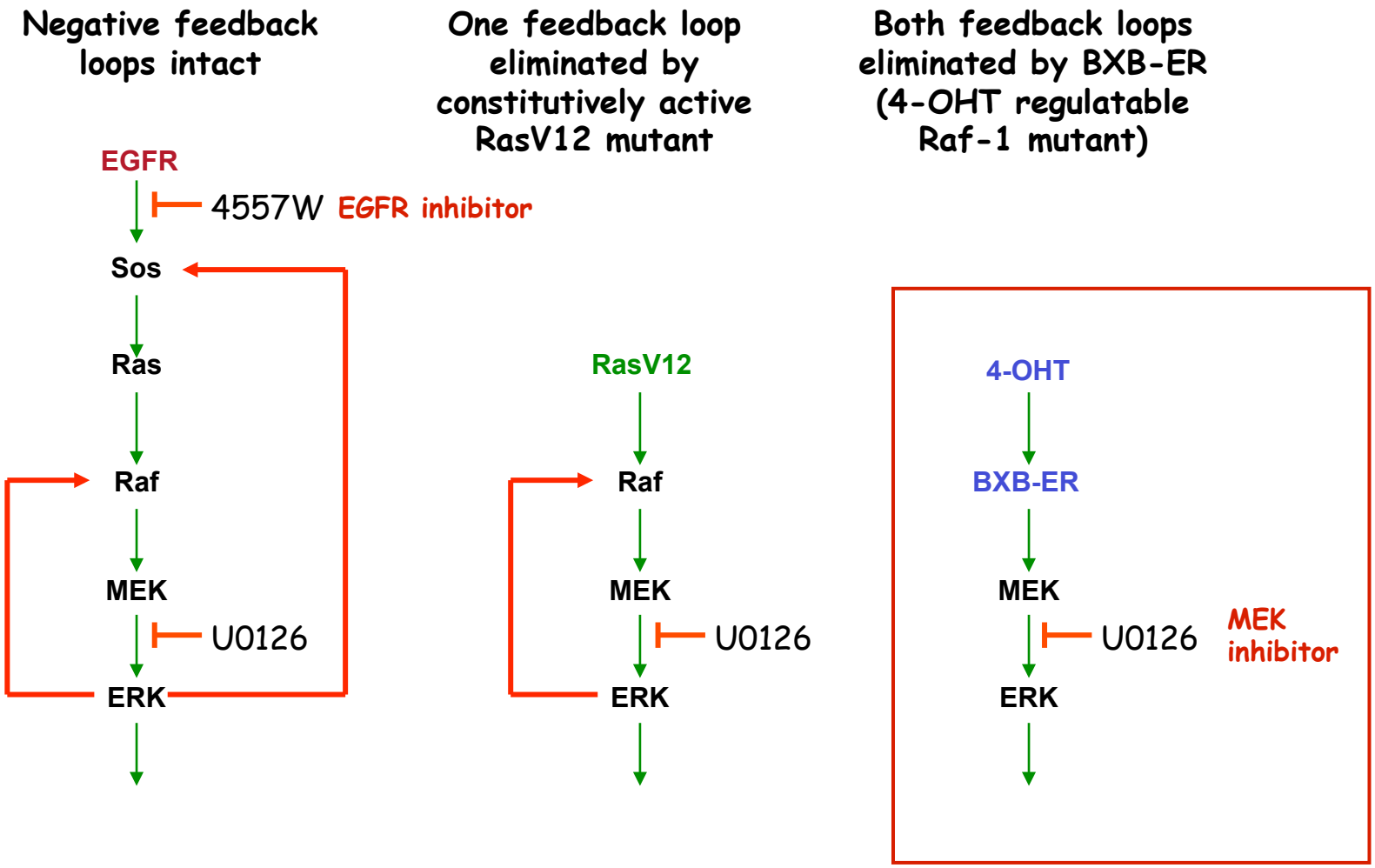
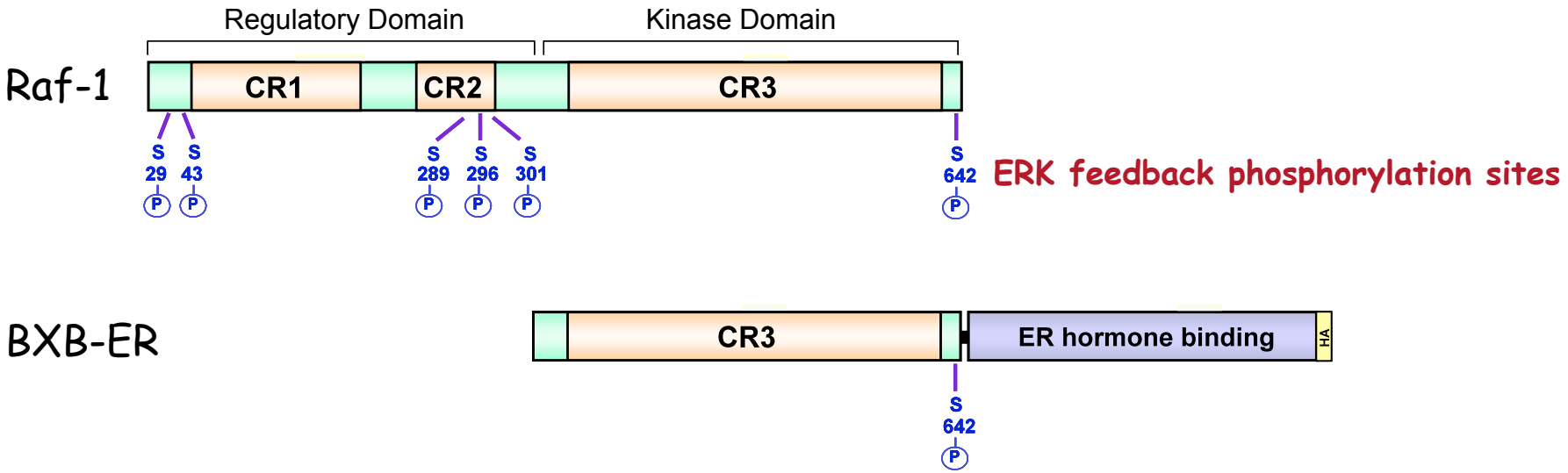
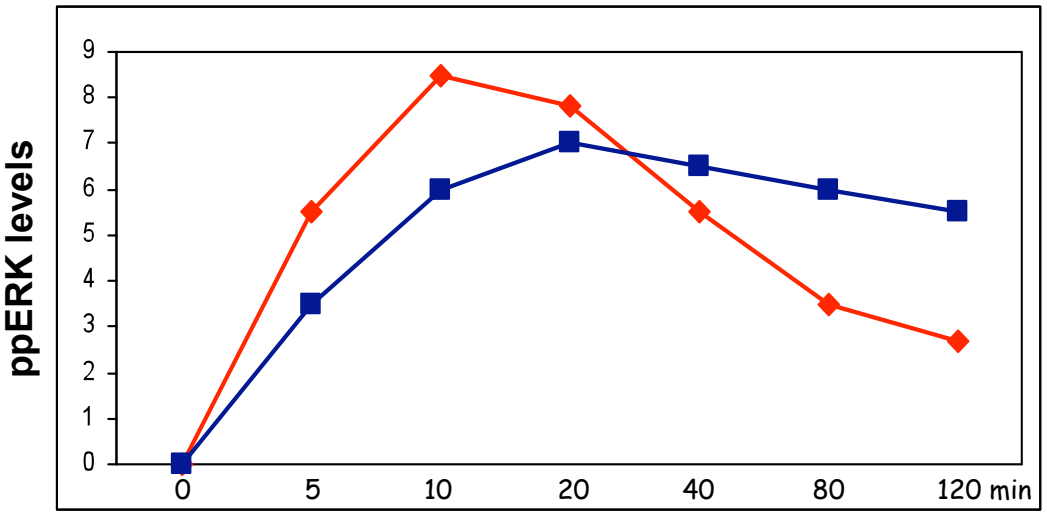
The experimental systems



Breaking the ERK feedback with BXBER

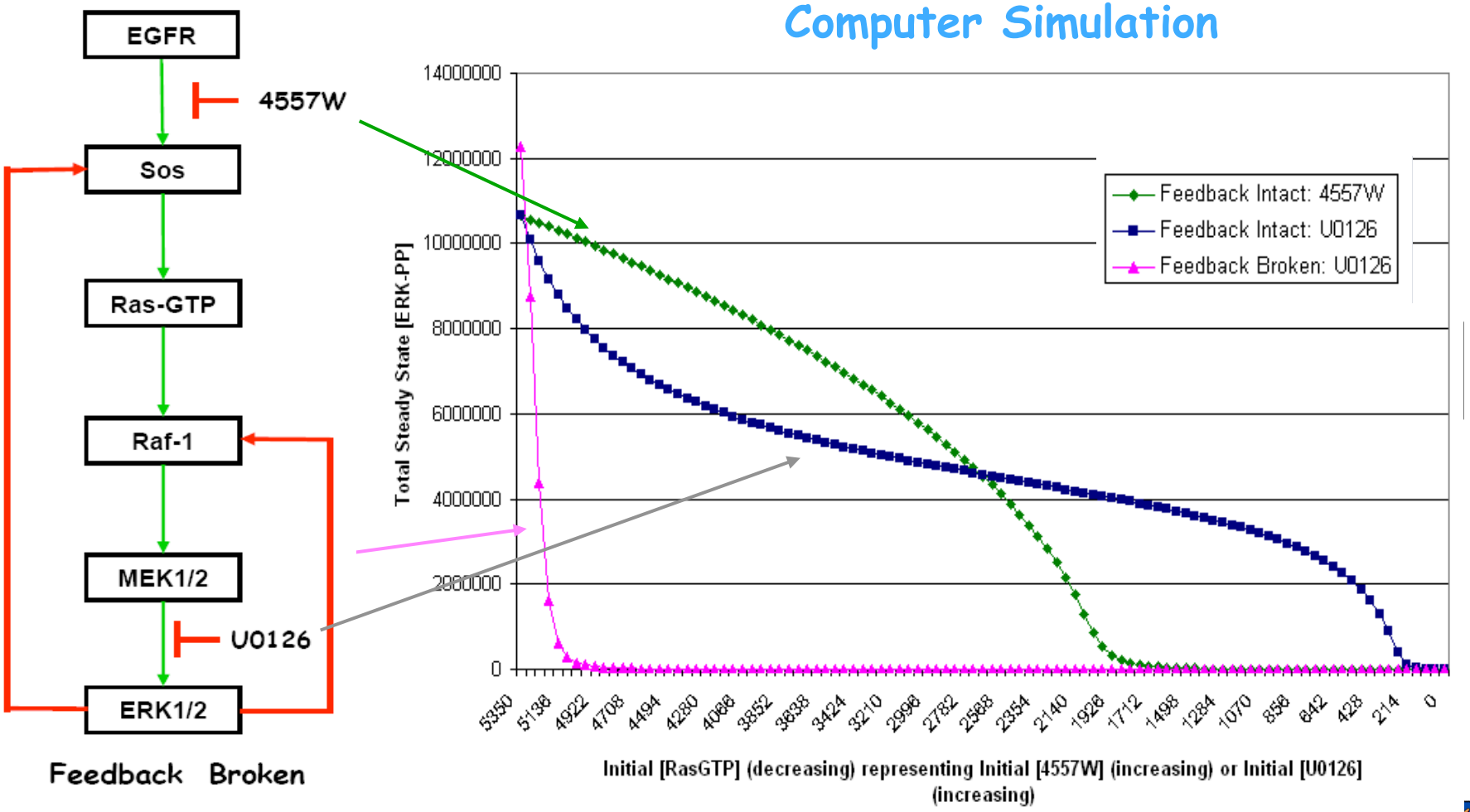




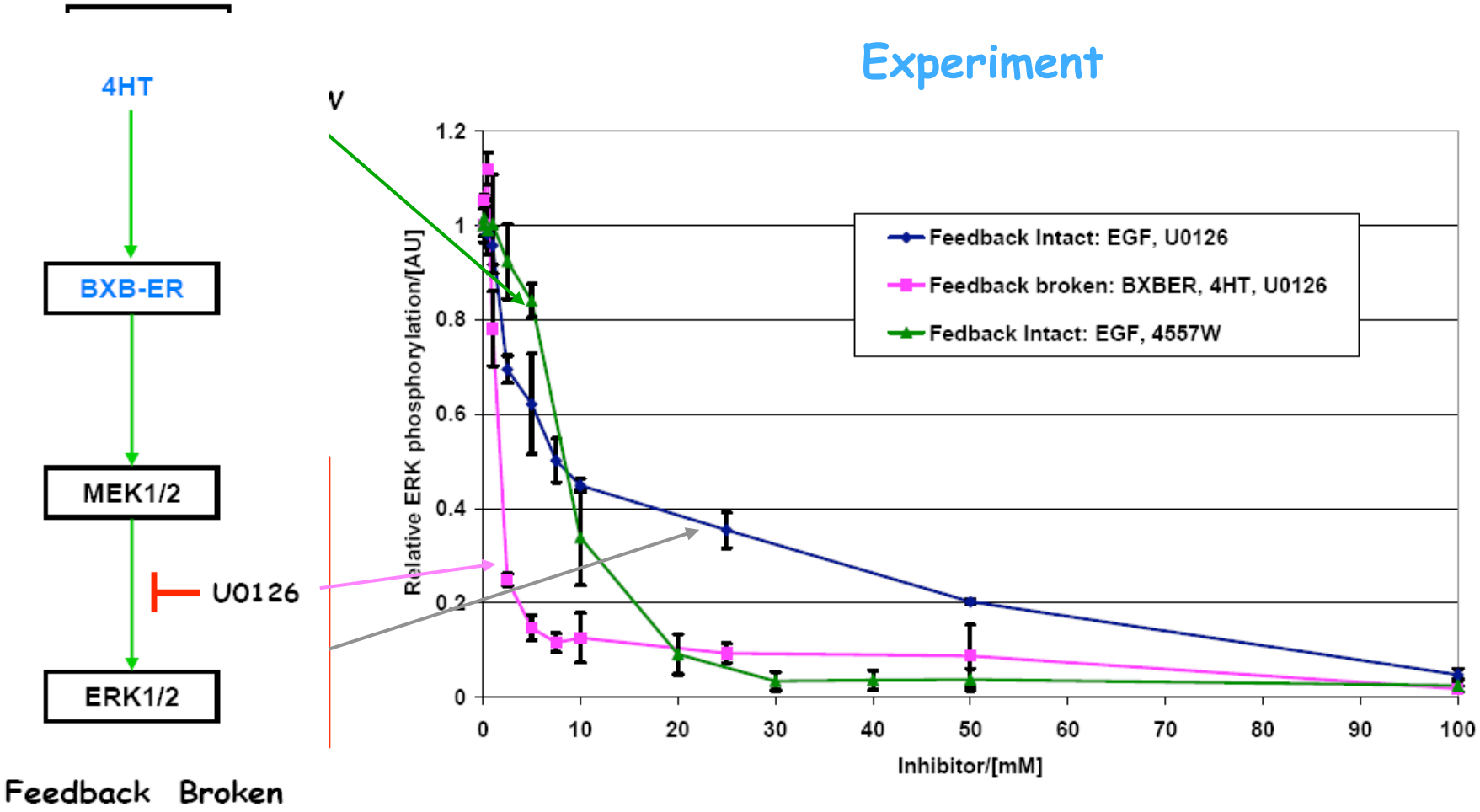
BXB-ER stimulated with 4-OHT
(4-Hydroxy Tamoxifen, a synthetic estrogen)

Raf-1 stimulated with EGF

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



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



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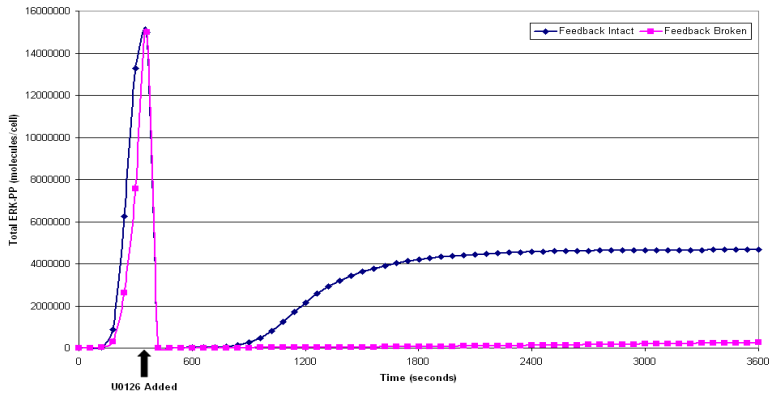
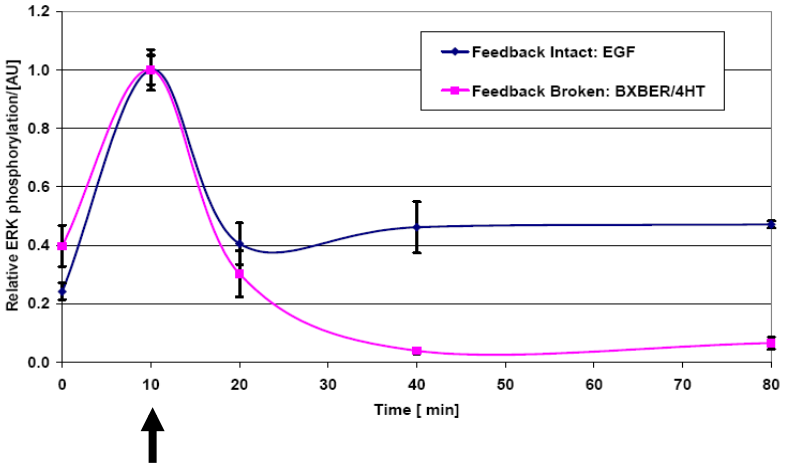
Signal recovery after MEK inhibition

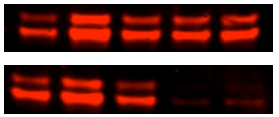
Simulation



Experiment



U0126 added



pERK1/2, +EGF

pERK1/2, + BXBER/4HT

0 10 20 40 80 min stimulation

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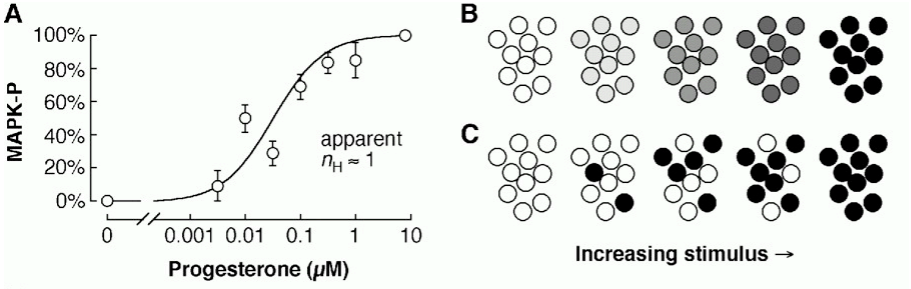
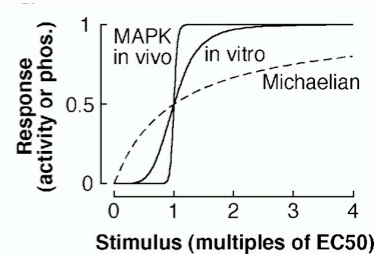
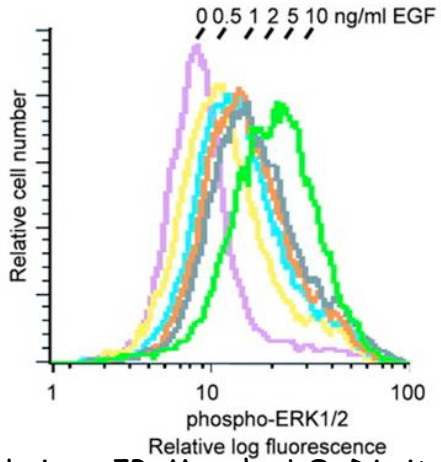
A current dispute: Does ERK activation follow graded or hypersensitive kinetics?

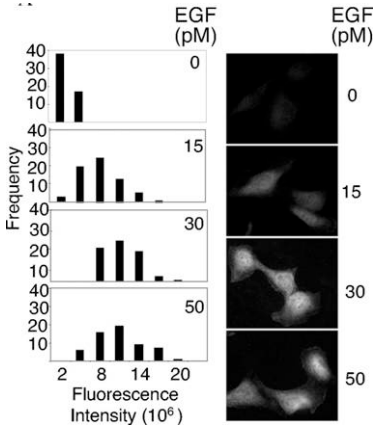




Ferrell JE Jr, Machleder EM.The biochemical basis of an all-or-none cell fate switch in Xenopus oocytes. Science. 1998 May 8;280(5365):895-8

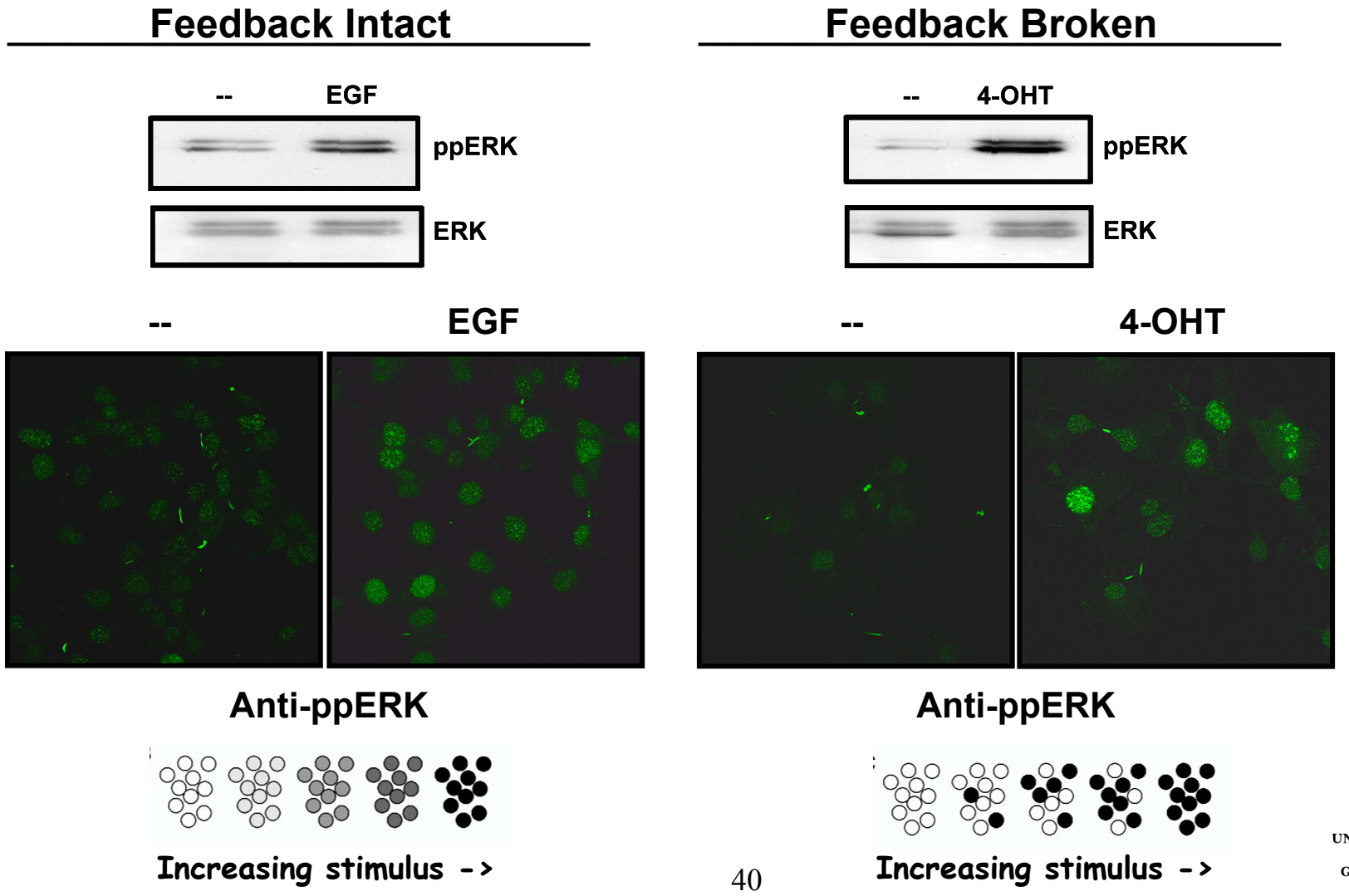


Mackeigan JP, Murphy LO, Dimitri CA, Blenis J. Graded mitogen-activated protein kinase activity precedes switch-like c-Fos induction in mammalian cells. Mol Cell Biol. 2005 Jun;25(11):4676-82.



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..



Implications for drug targeting

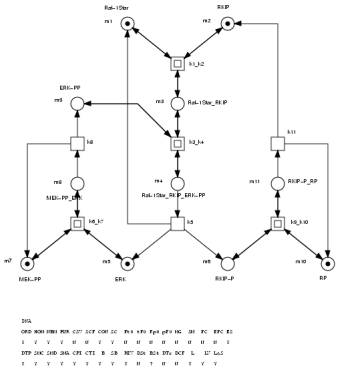
- The aim of a drug is to cause a disruption to the network in such a way that it restores the network to its ‘healthy’ wild-type state.
- Targets must be susceptible to disruption for the drug to have any effect.
- The analysis of feedback suggests that targets inside the feedback loop will prove difficult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

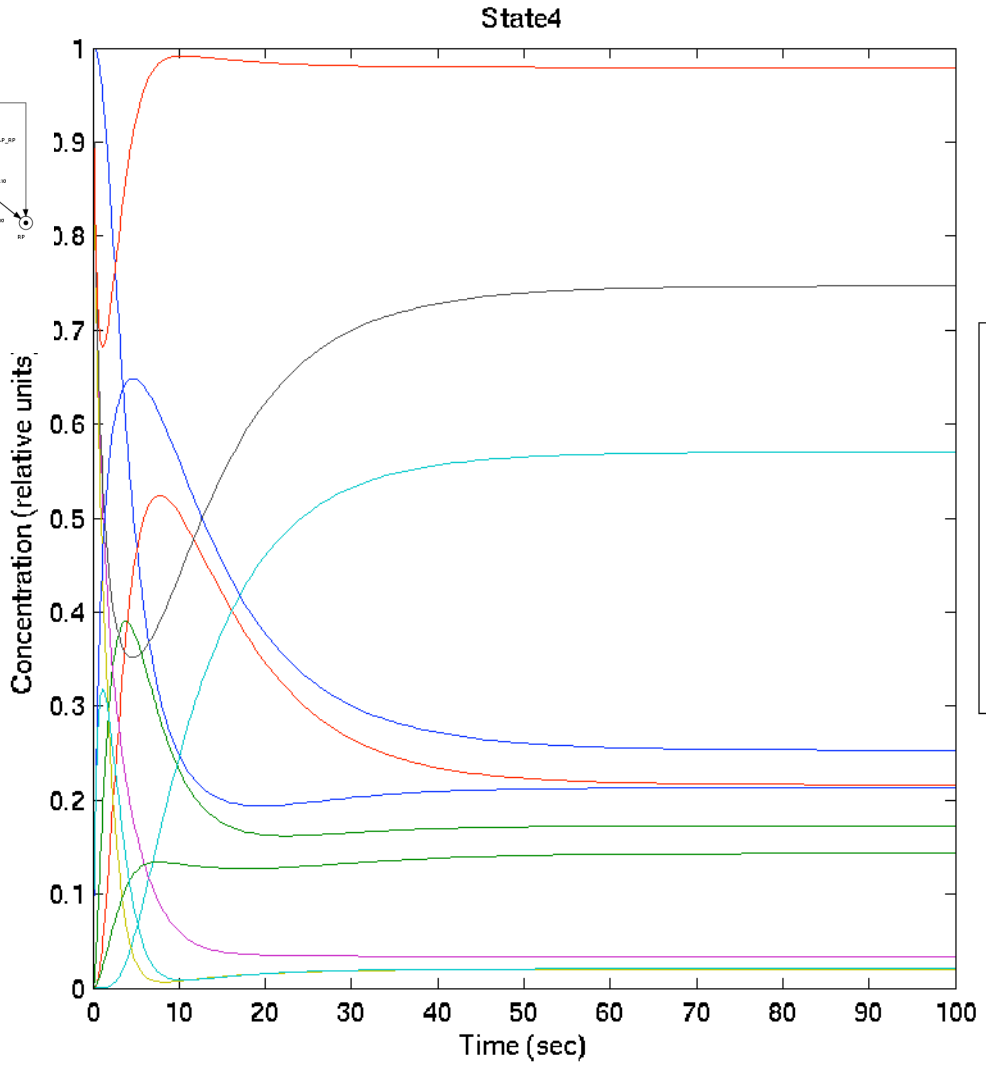
Take home messages

- Modelling can be done using
  - Michaelis Menten
  - Mass Action
- Some difference in results using different models
- Wet-lab results show clear negative feedback amplifer characteristics

From Petri Nets to Differential Equations - an Integrative Approach

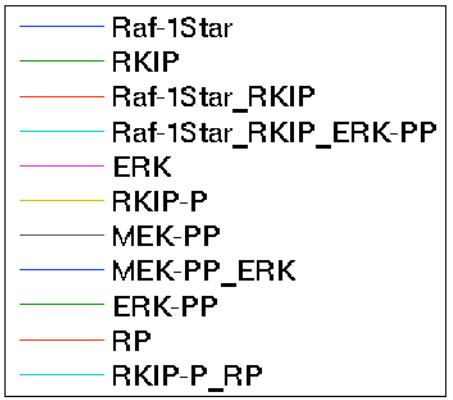
David Gilbert & Monika Heiner

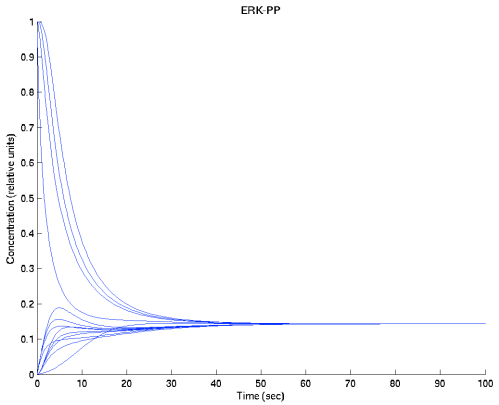




Species	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13
Raf-1*	1	0	0	1	1	1	1	1	0	0	1	1	1
RKIP	1	0	0	0	0	0	0	1	0	0	1	0	0
Raf-1*	0	1	0	0	0	0	0	0	1	1	0	0	0
Raf-1*/RKIP/ERK-PP	0	0	1	0	0	0	0	0	0	0	0	0	0
ERK	0	0	0	1	0	0	1	1	1	0	0	0	0
RKIP-P	0	0	0	1	1	0	0	0	0	0	0	0	1
MEK-PP	1	1	1	1	0	0	1	1	1	0	0	1	1
MEK-PP/ERK	0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

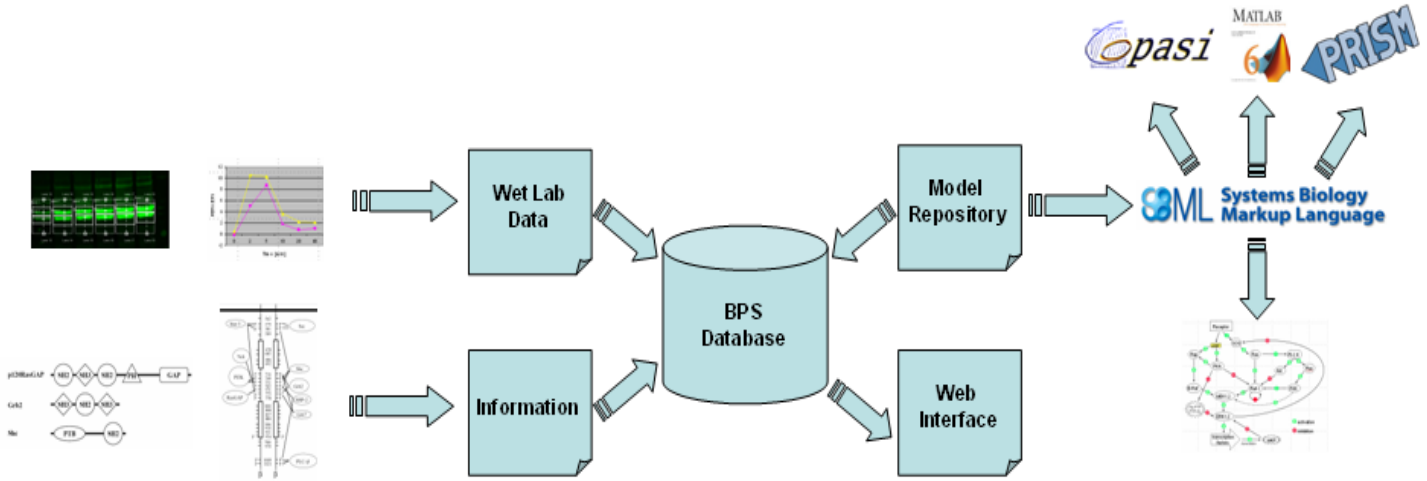




BPS Database

Richard Orton

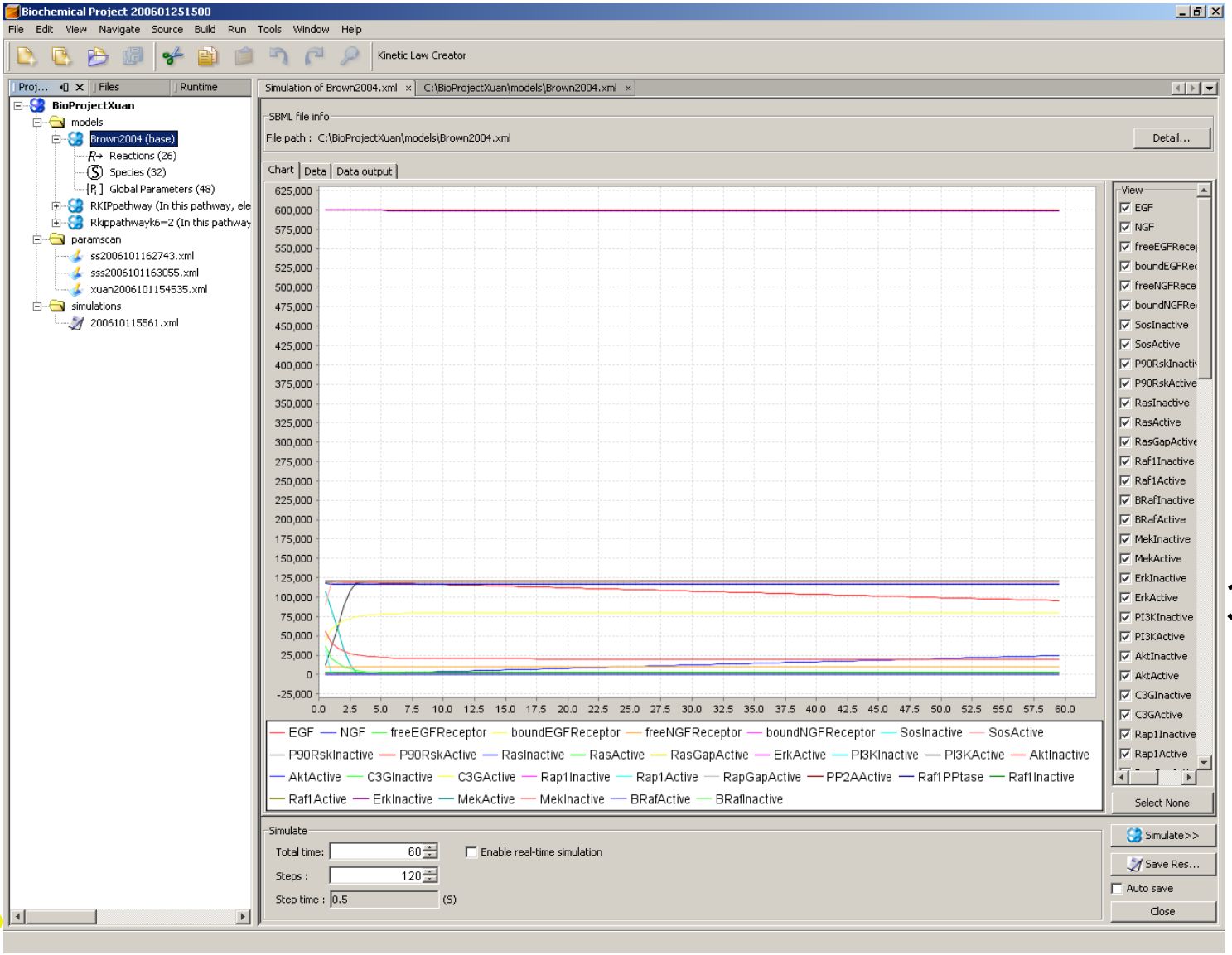
- We are developing a database to store a variety of data generated from the project
  - biochemical models complete with parameter data
  - wet lab modelling data
  - biochemical information on the pathways and proteins involved



- We are also developing a number of software tools to aid in the modelling process:
  - Database import/export
  - Model visualisation
  - Model conversion

The BPS Software Workbench

Xuan Liu, Vladislav Vyshemirsky and David Gilbert



Cluster

GRID

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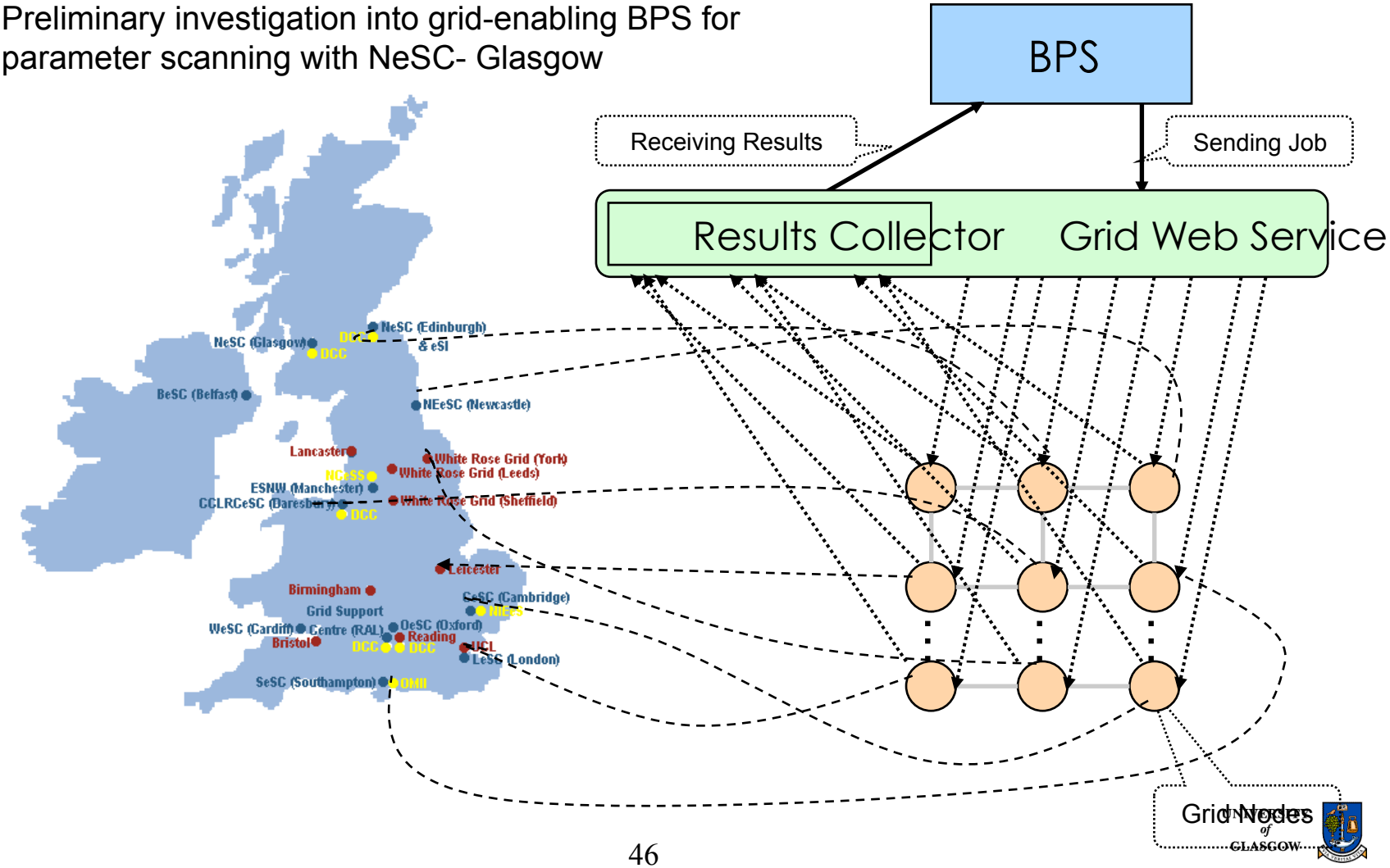
BPS on the Grid

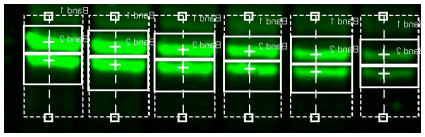
- Preliminary investigation into grid-enabling BPS for parameter scanning with NeSC- Glasgow



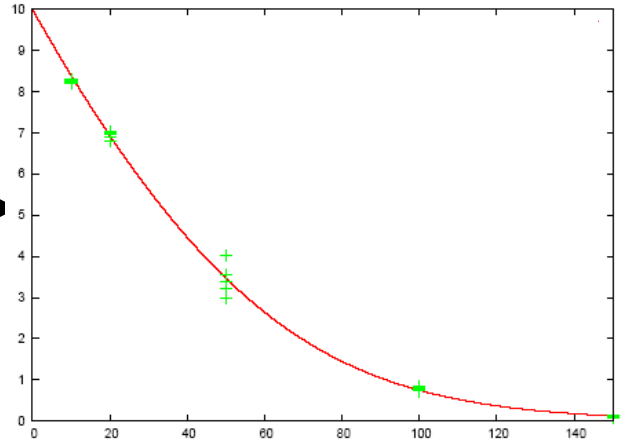
Using Bayesian Inference to I model parameters

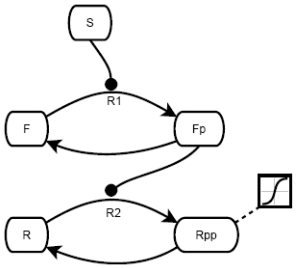
Vladislav Vyshemirsky & Mark Girolami

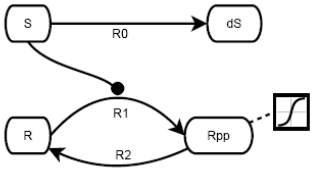
Lab data

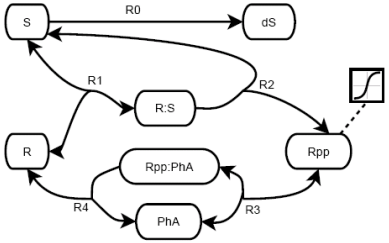


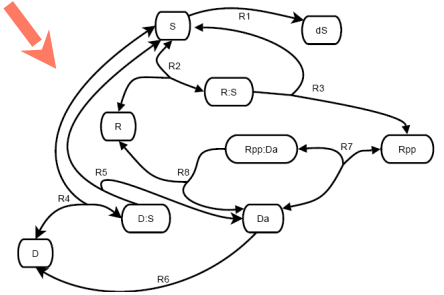
Parameter distributions











Model inference
Rate parameters
&
topologies

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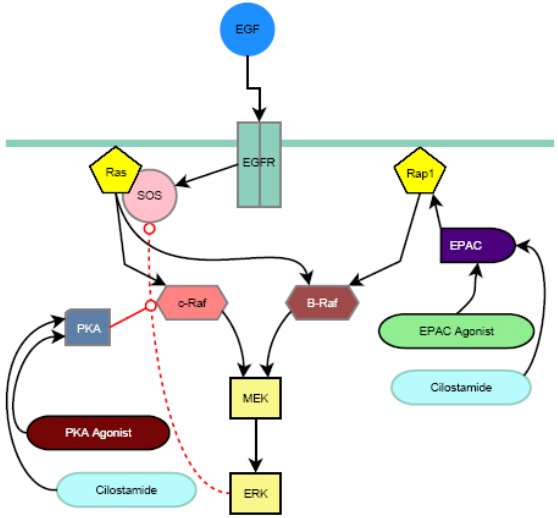
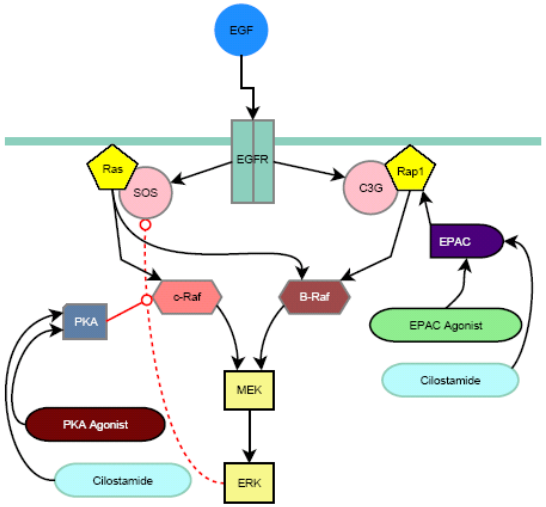
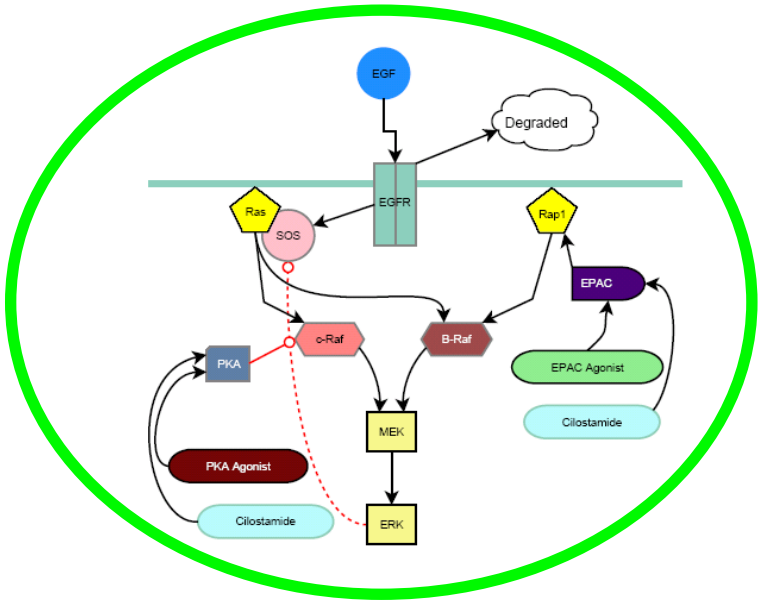
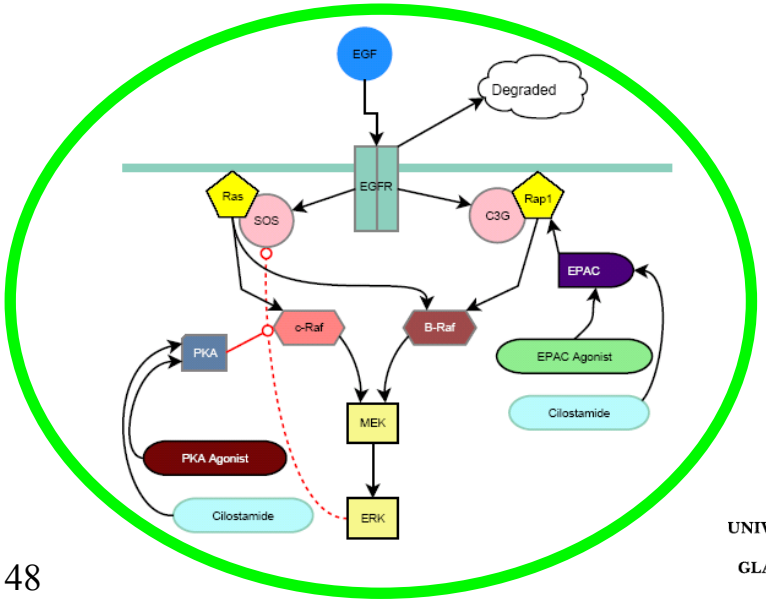
Model Comparison









Acknowledgements

DTI Beacon Project Biological Pathway Simulator and Analyser

David Gilbert, **Walter Kolch**, Xu Gu, Muffy Calder, Richard Orton, Amelie Gormand, Tamara Polajnar, Vlad Vyshemirsky, Oliver Sturm, Xuan Liu, Mark Girolami

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

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

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