Modelling the kinetic behaviour of the MAPK cascade:

an example of the potential of systems biology as a tool to aid drug discovery

David Gilbert

Bioinformatics Research Centre,
University of Glasgow

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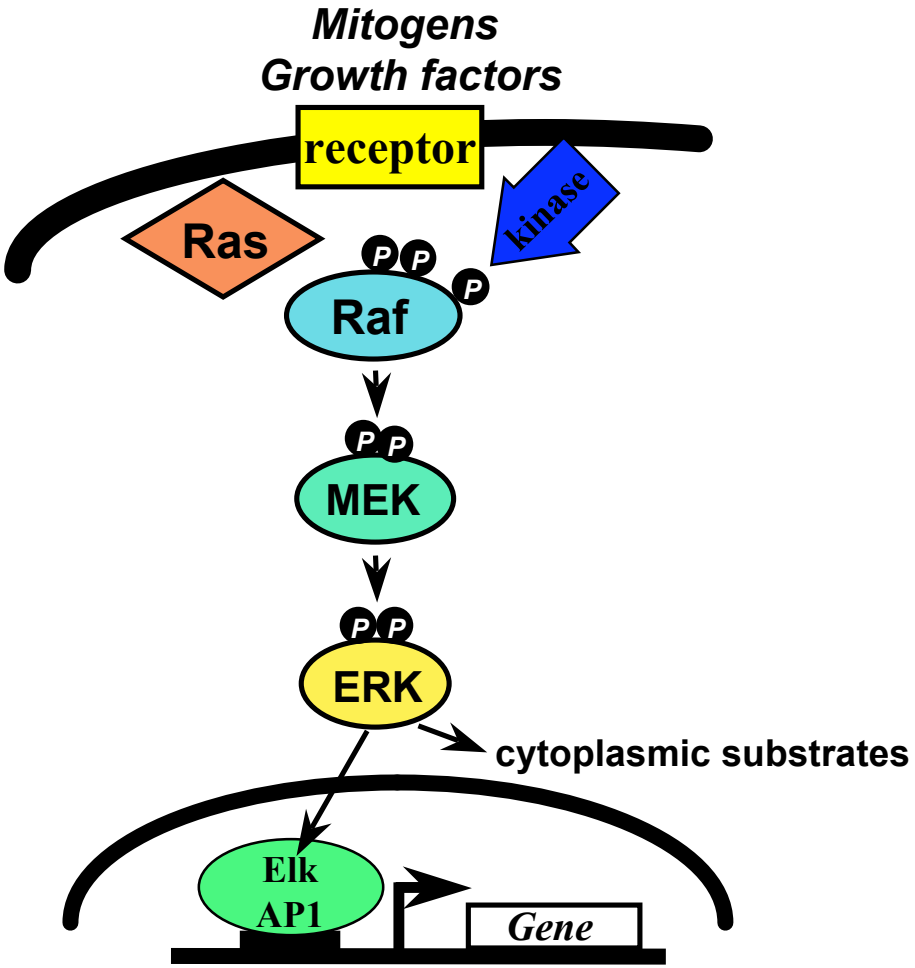
The Ras – Raf – MEK – ERK Signalling pathway



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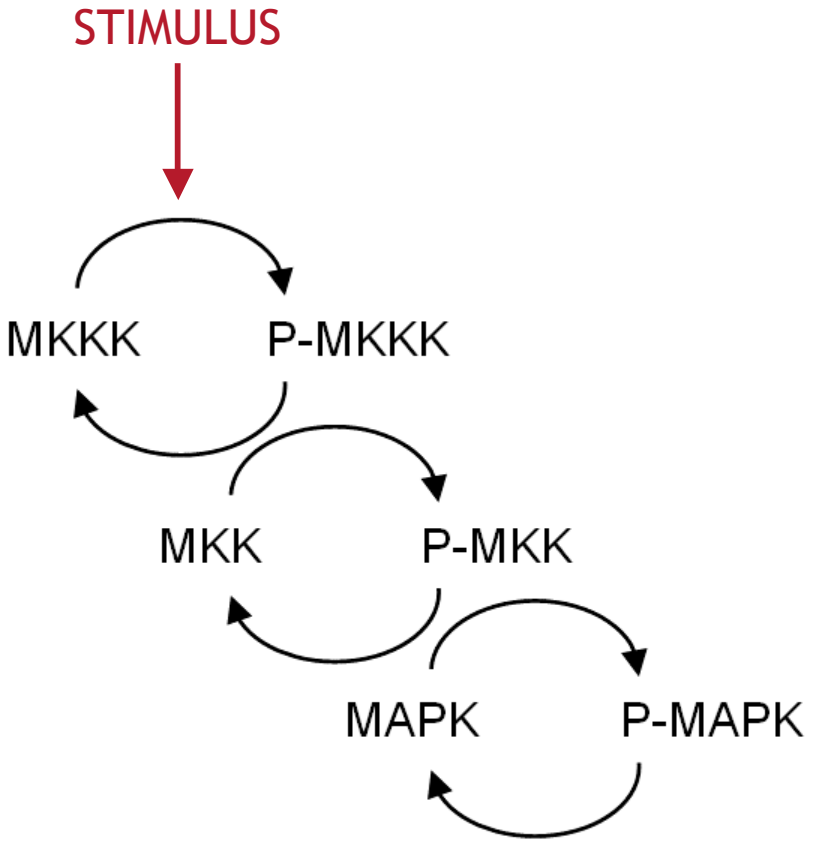
MAPK Pathway

- 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.



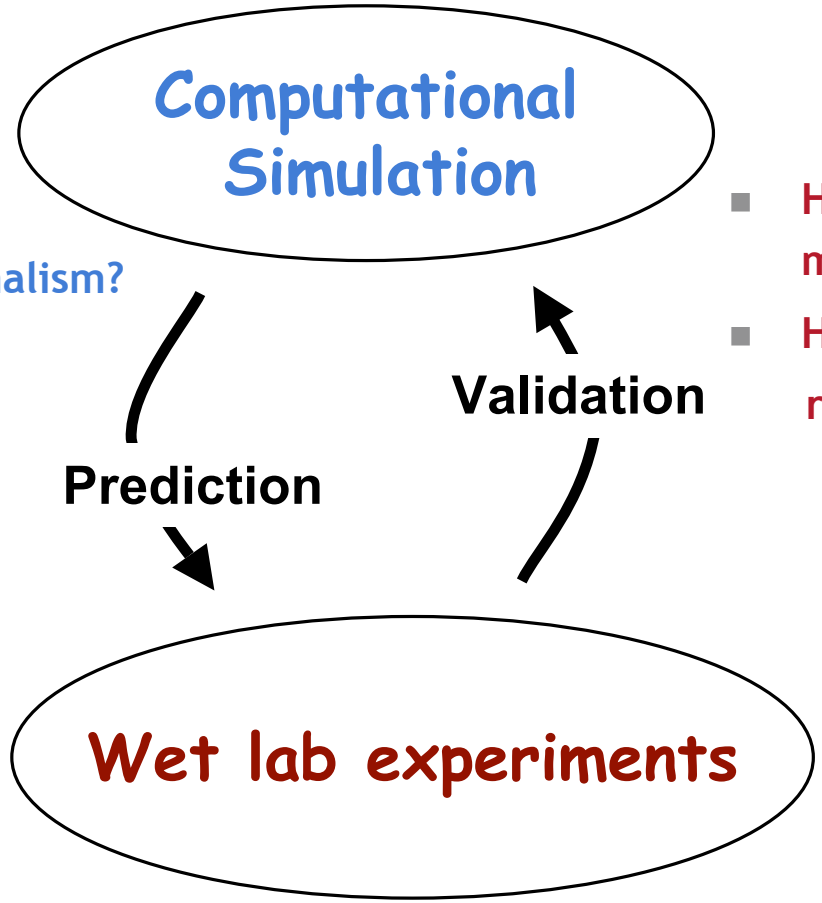
But…

- Current drugs aimed at decreasing the activity of the MAPK pathway (e.g. U0126)

  have proved less efficient in *in vivo* applications than anticipated from *in vitro* inhibition assays

Biochemical Pathway Simulation (BPS)- project:

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



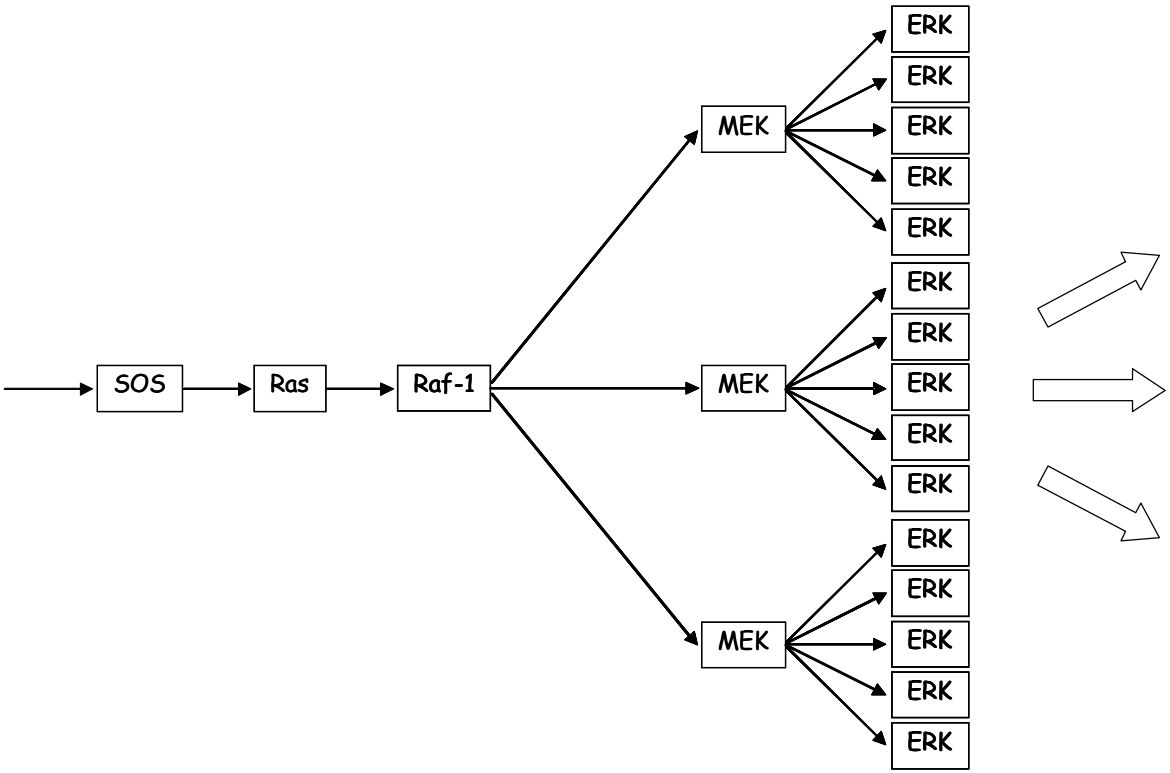
- How to collect quantitative measurements in vivo?
- How to manipulate regulatory mechanisms?

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.

Amplification

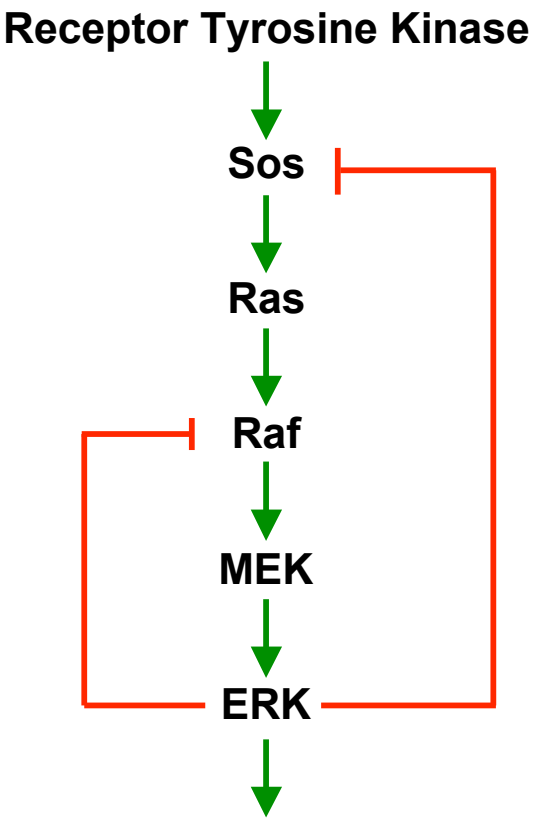
- ERK cascade well known biological amplifier -- amplifies the original signal to create effective cellular responses.
- 1:3:5 are the approximate ratios of Raf-1, MEK and ERK in fibroblasts.



Negative Feedback

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

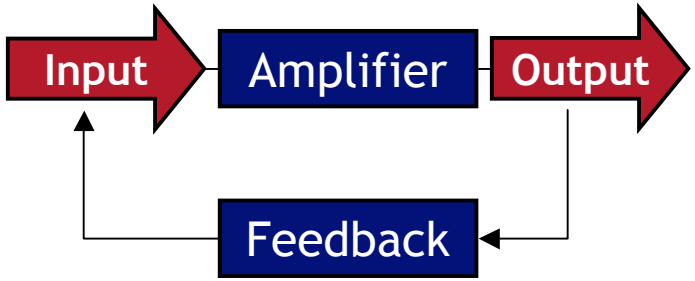
- New negative feedback loop: ERK-PP phosphorylates Raf-1 resulting in a hyper-phosphorylated inactive form of Raf (Dougherty *et al*. 2005)



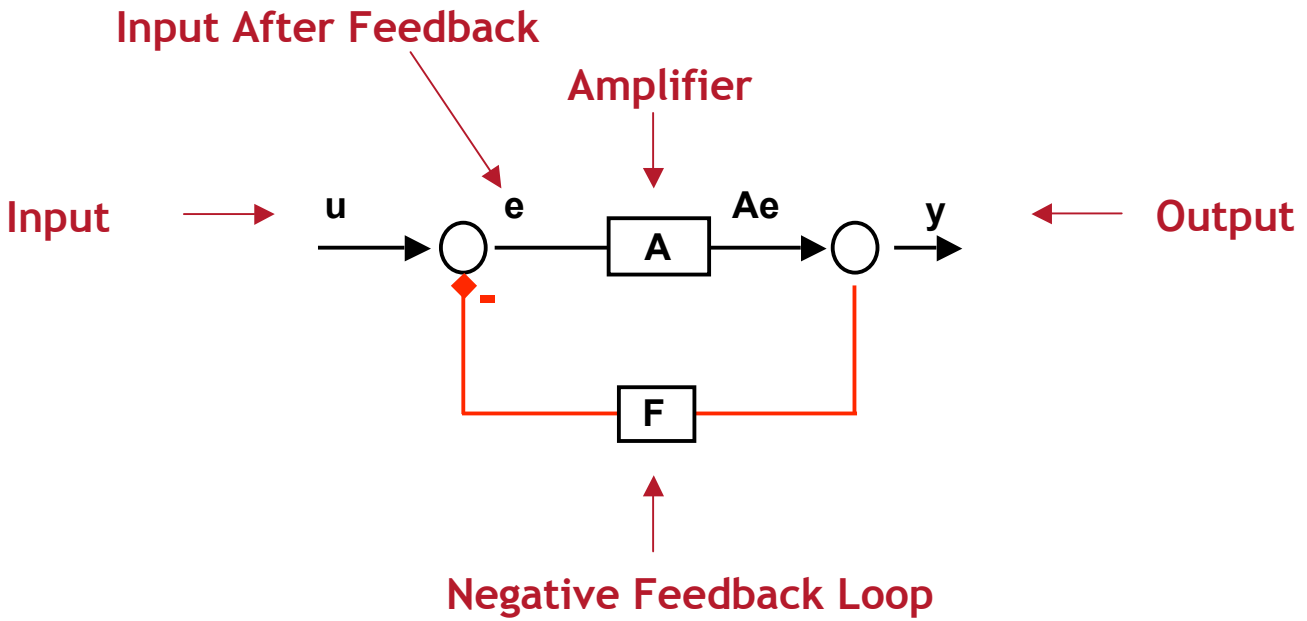
Dougherty *et al*. (2005), Regulation of Raf-1 by Direct Feedback Phosphorylation, Molecular Cell 17 215-224

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



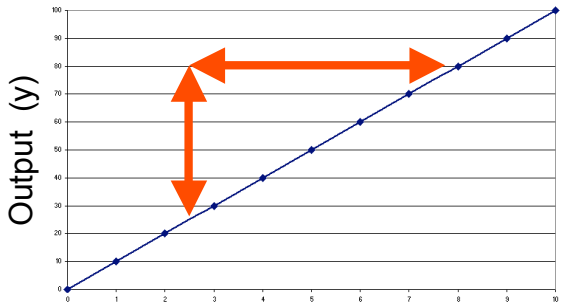
Steady State Equation $$y = \frac{Au}{1 + AF}$$

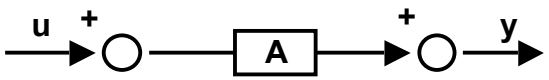
y = Ae
e = u - Fy

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

The negative feedback imparts signalling robustness

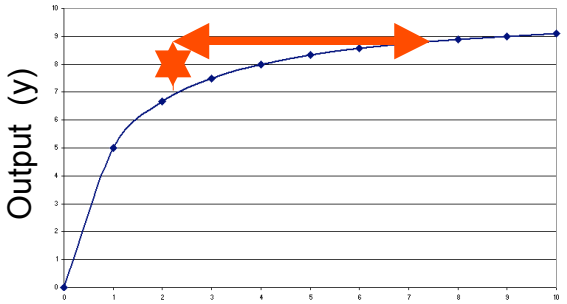
Standard Amplifier

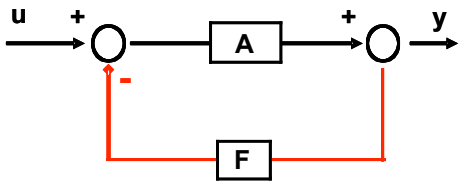




y=A*u

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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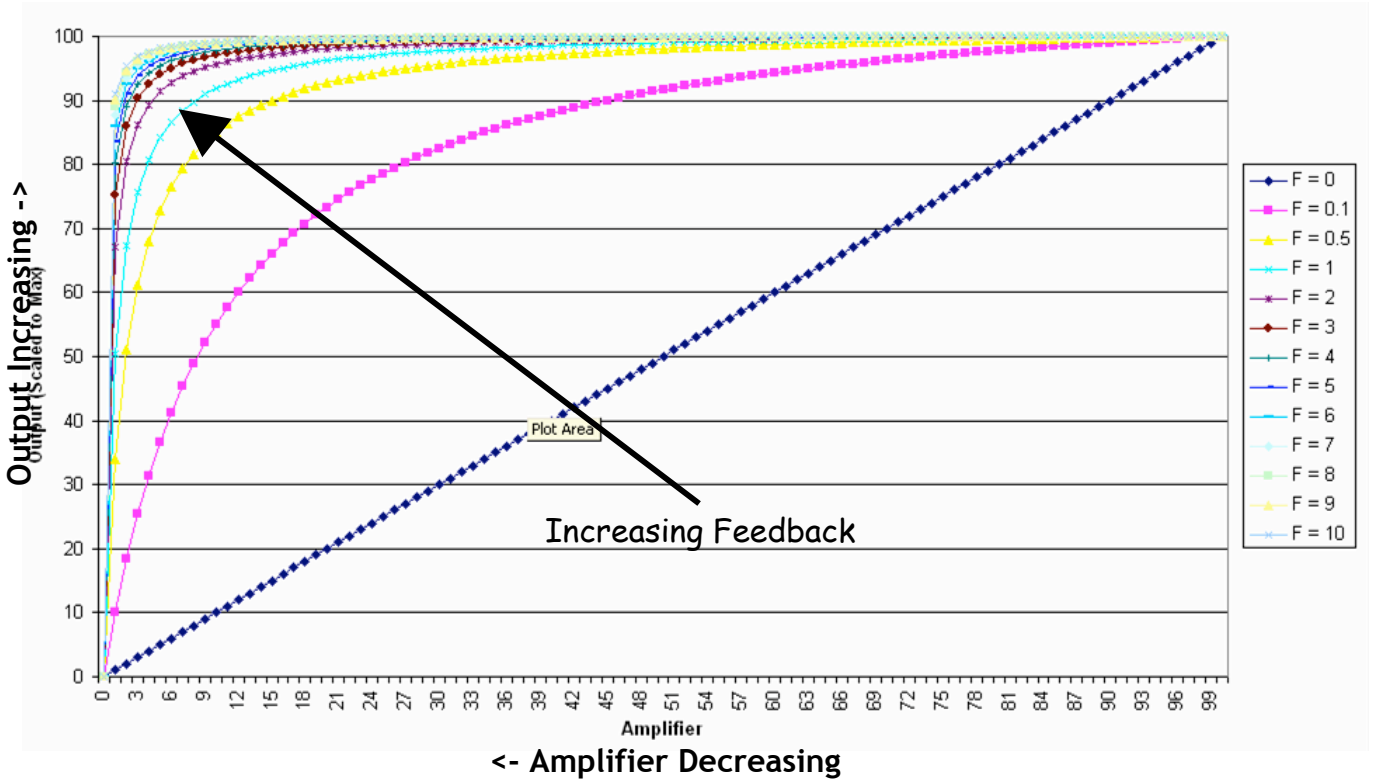
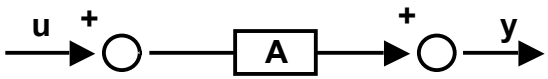
Feedback


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

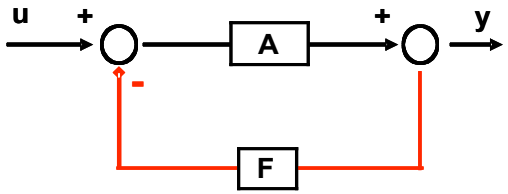
The negative feedback imparts signalling robustness

Standard Amplifier



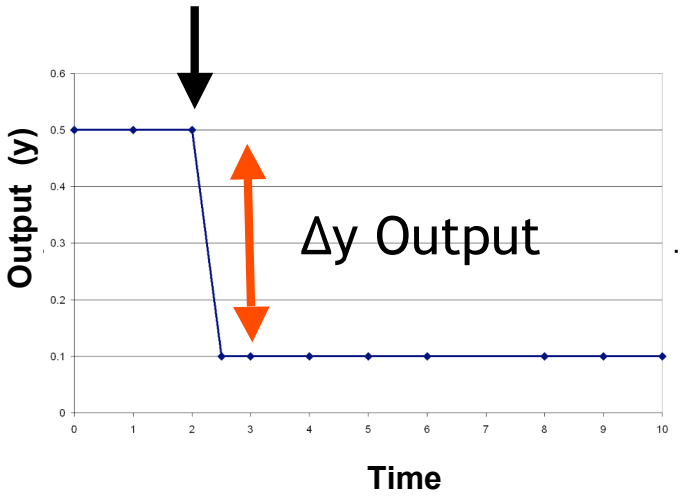
y=A*u

Negative Feedback Amplifier

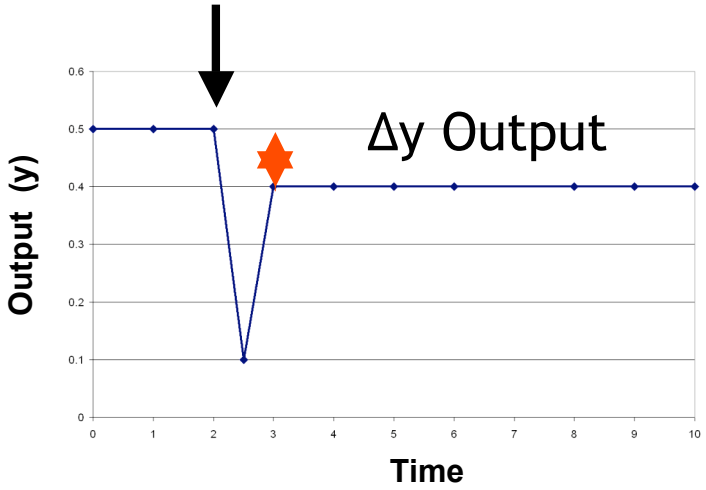


y=A*u/(1+A*F)

Sudden drop in Amplifier (A) gain

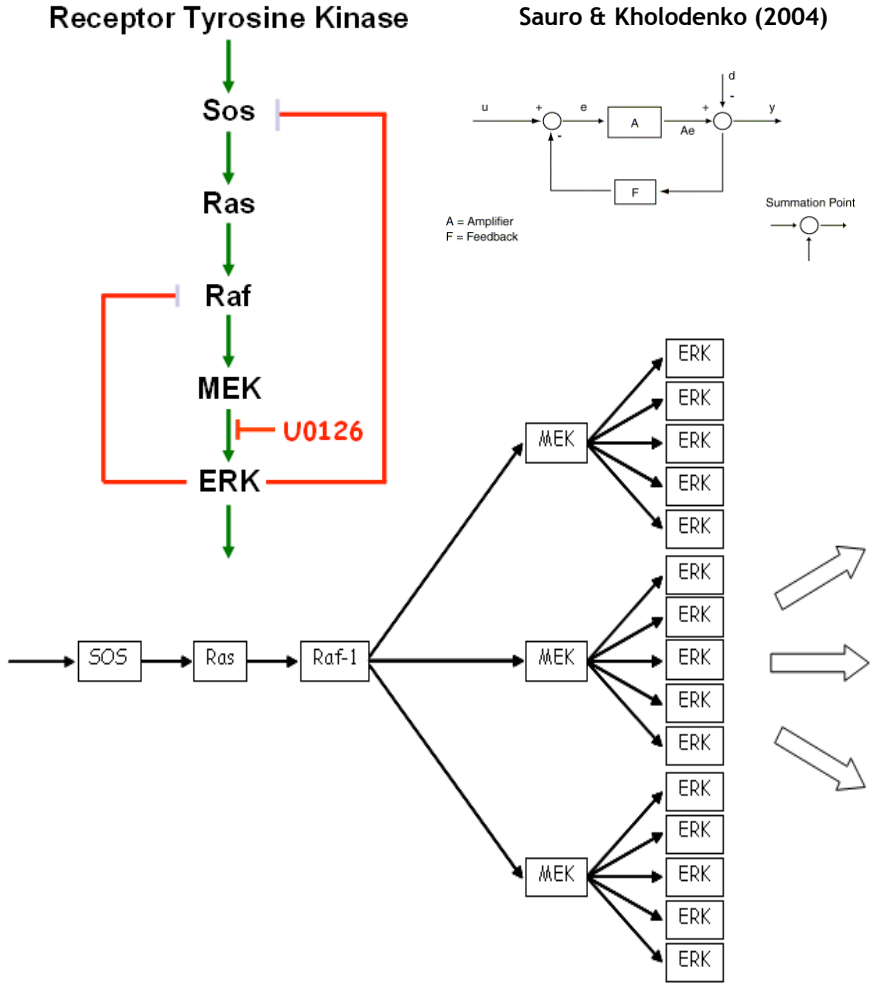


Sudden drop in Amplifier (A) gain

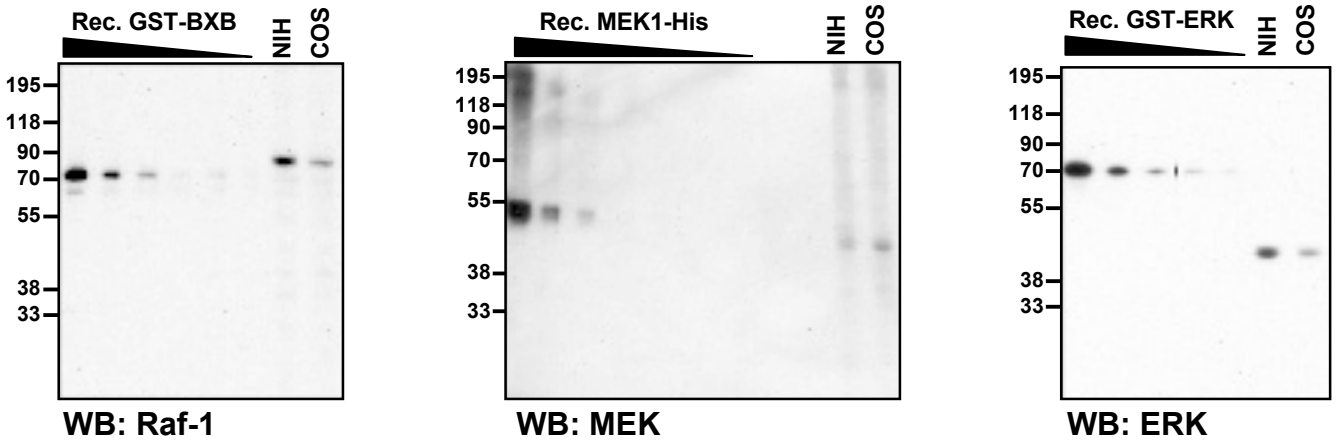


Application to Biology

- 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.



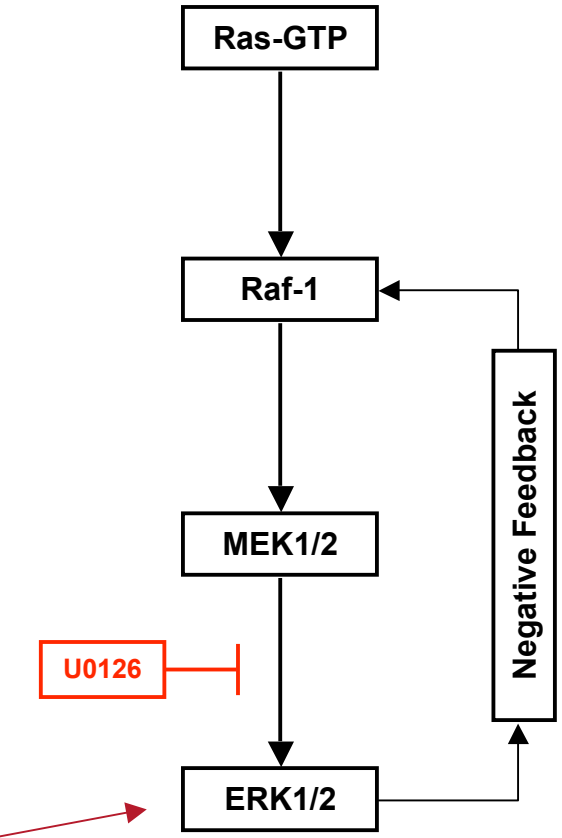
Raf/MEK/ERK amplifies the signal



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



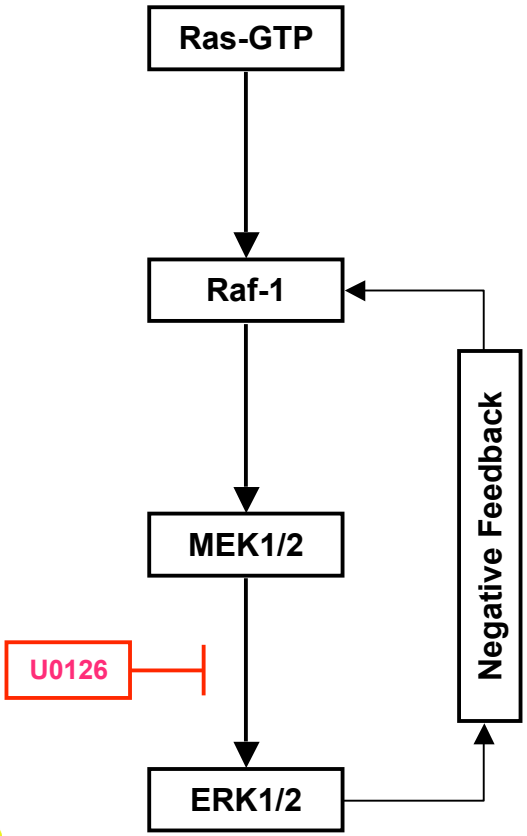
Remove negative feedback

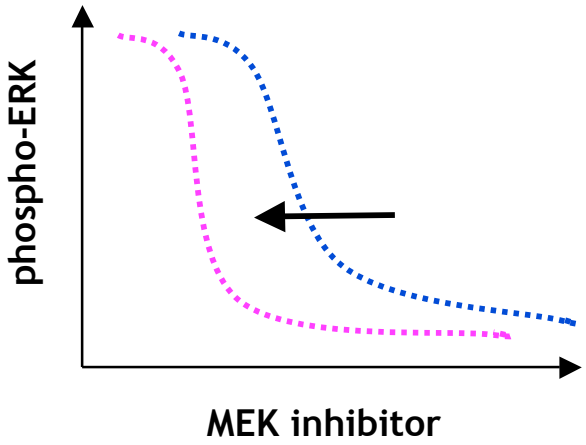
"Disturb the Amplifier":
Use a MEK inhibitior, such as U0126

Measure signal output:
i.e. ERK phosphorylation

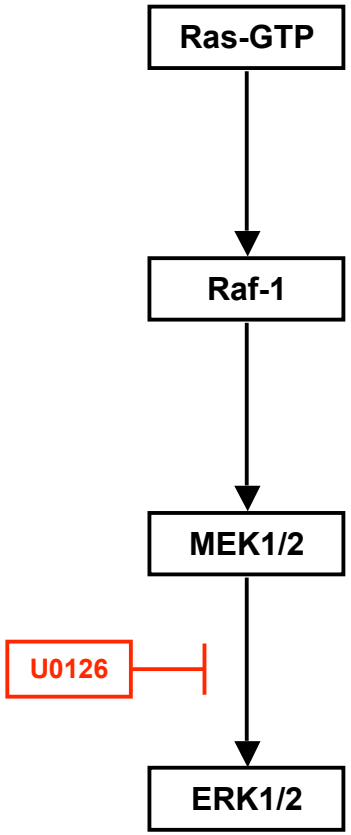
Hypothesis: Braking the feedback should sensitise the ERK pathway to MEK-inhibitor

Feedback intact





Feedback removed



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

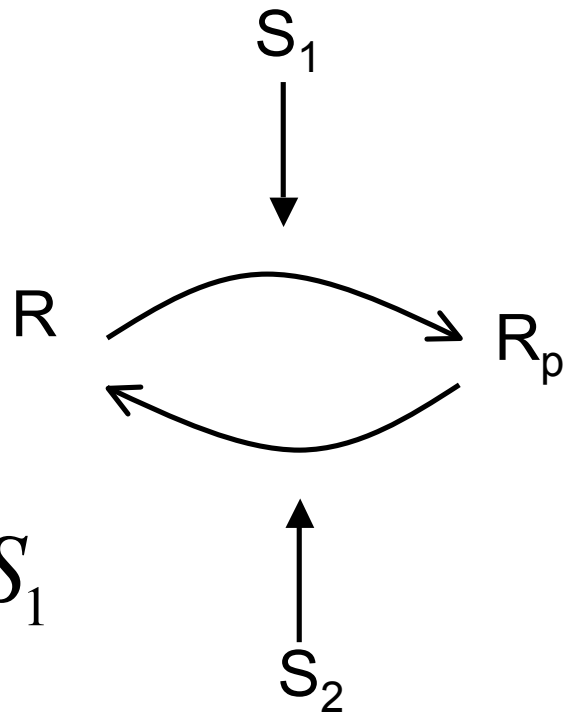
Strategy

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

Computational Model of ERK pathway with/without feedback

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 cascade: 3-stage, Mass-Action model 1

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

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

$$SS_1 = R_p$$

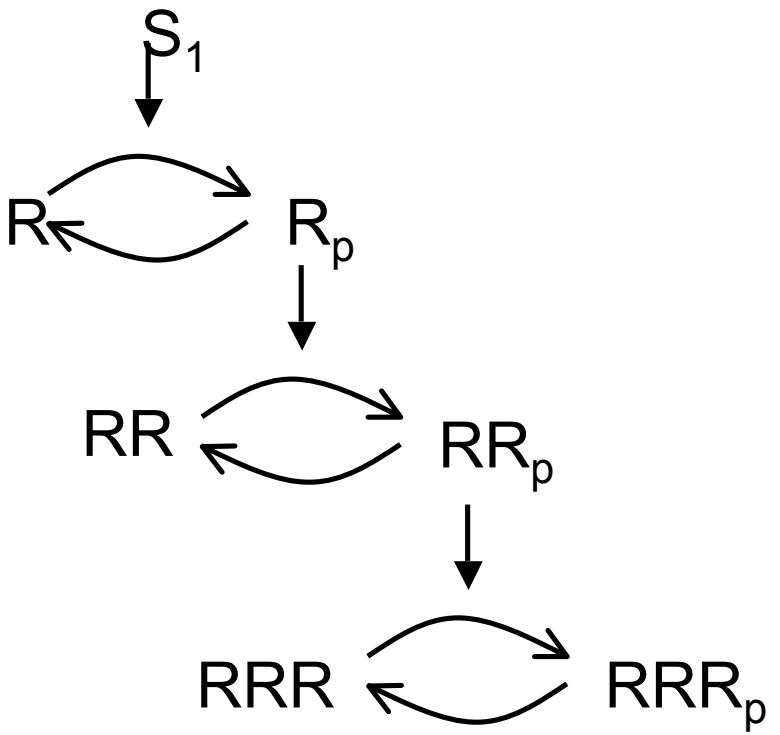
$$RR + SS_1 \underset{kk_2}{\overset{kk_1}{\rightleftarrows}} RR\,|\,SS_1 \xrightarrow{kk_3} RR_p + SS_1$$

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

$$SSS_1 = RR_p$$

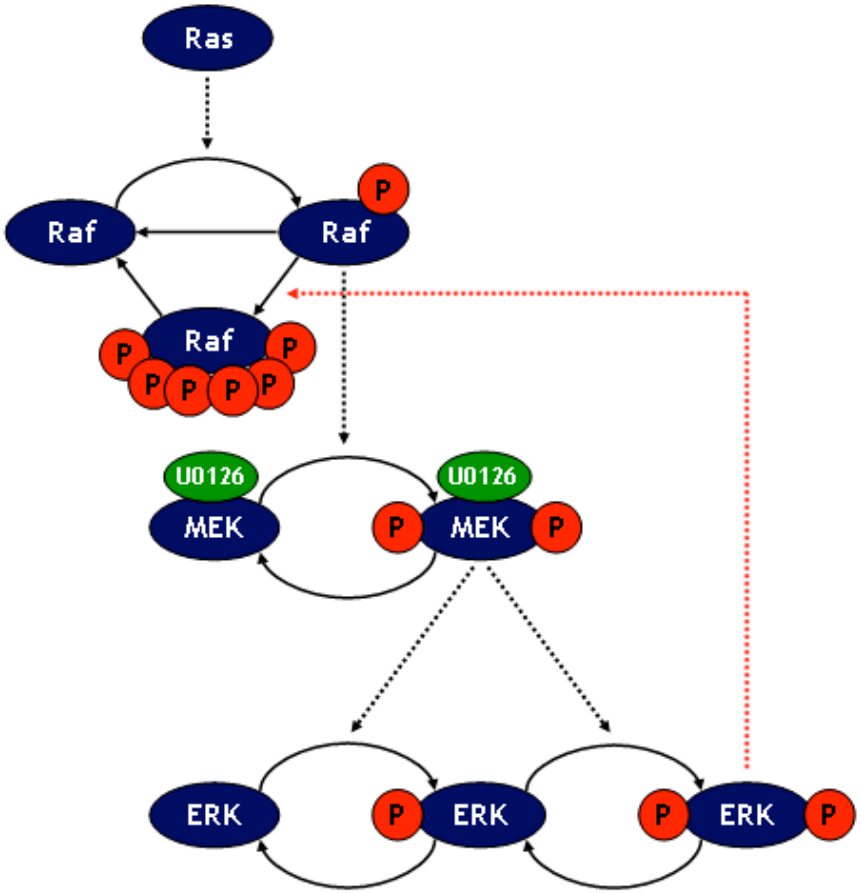
$$RRR + SSS_1 \underset{kkk_2}{\overset{kkk_1}{\rightleftarrows}} RRR\,|\,SSS_1 \xrightarrow{kkk_3} RRR_p + SSS_1$$

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



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"

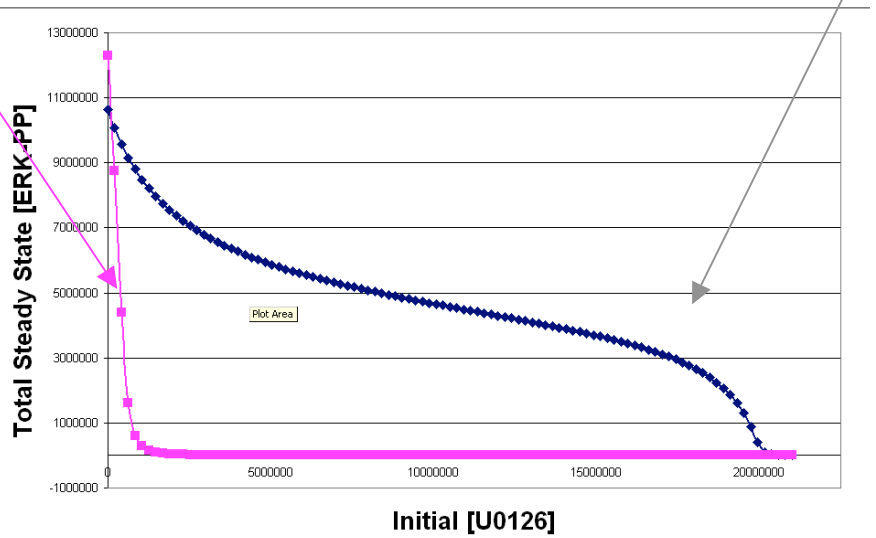


Schoeberl *et al.* (2002), Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors, Nature Biotechnology 20, 370-375

Computational Modeling 2: Results

Feedback broken

Feedback intact



Prediction: Braking the feedback modulates drug response

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The experimental systems

(1) Negative feedback loops intact

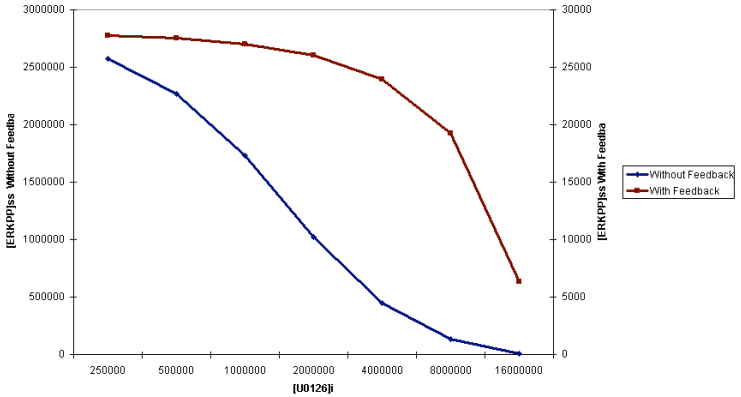
(2) One feedback loop eliminated by constitutively active Ras mutant

(3) Both feedback loops eliminated by 4-OHT regulatable Raf mutant

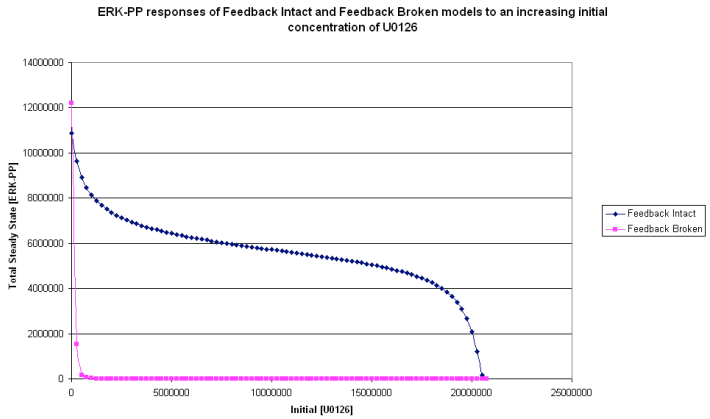
Non-Classical Behaviour?

- This is not the classical behaviour one would expect from a negative feedback amplifier system
- However, this is to be expected as the biological system is much more complex than the relatively simple electronic circuit, therefore it is not going to behave in exactly the same way.
- What is important is that there are very clear differences between the Feedback Broken and Feedback Intact models
- The Feedback Intact model is much much more resistant to the effects of U0126.

Classical Behaviour



Biological Behaviour



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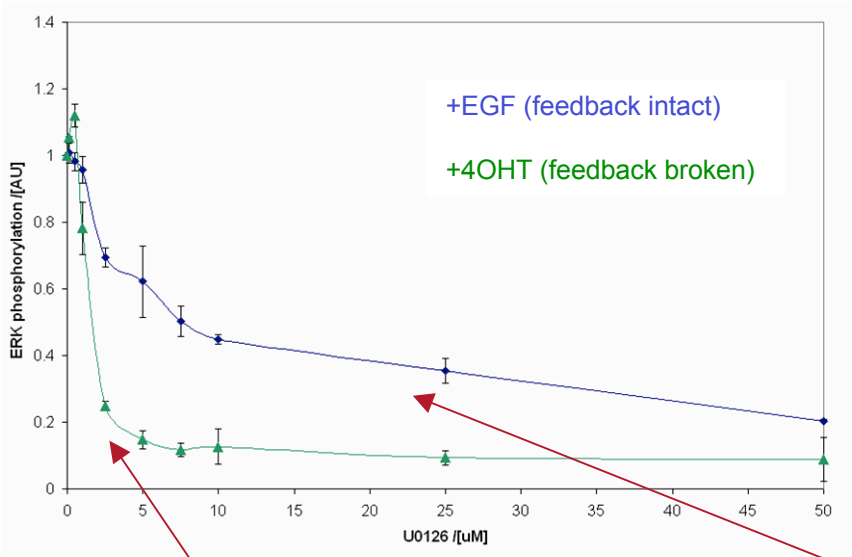
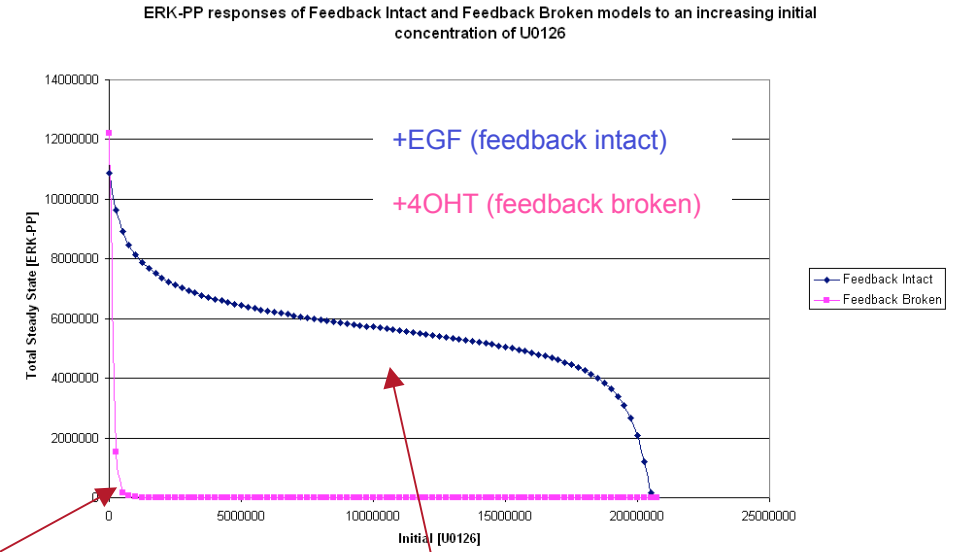
Comparison to Wet Lab data

LAB



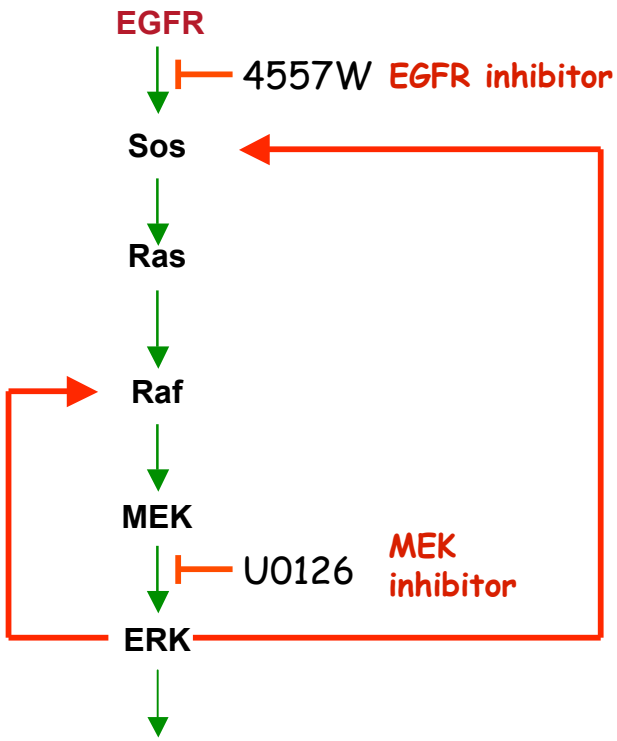
MODEL



Feedback Broken
ERK-PP levels rapidly decrease to basal levels

Feedback Intact
ERK-PP levels remain relatively high over a Broad concentration range
Both display initial sensitivity and then robustness

Biological Implications: Where to inhibit?

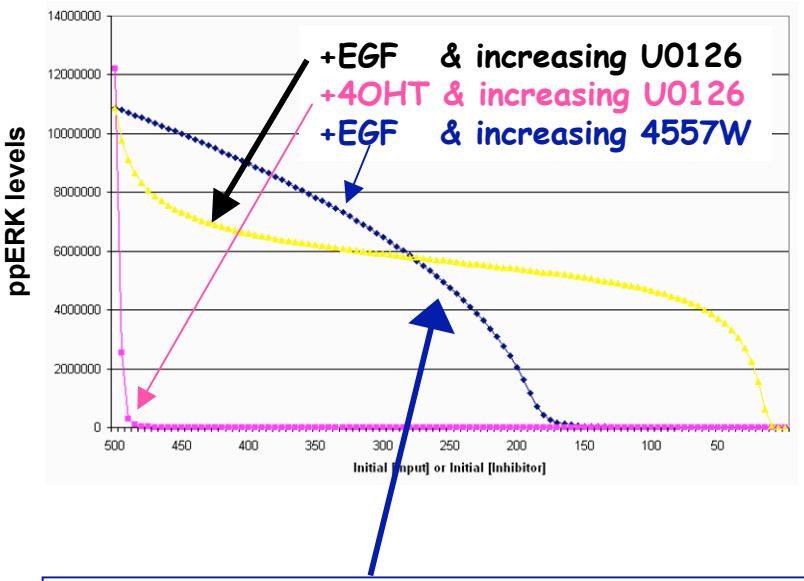


Our results suggest that drug targets outside of feedback loops are easier to hit and hence are preferable to drug targets embedded into feedback loops.

Is that so?

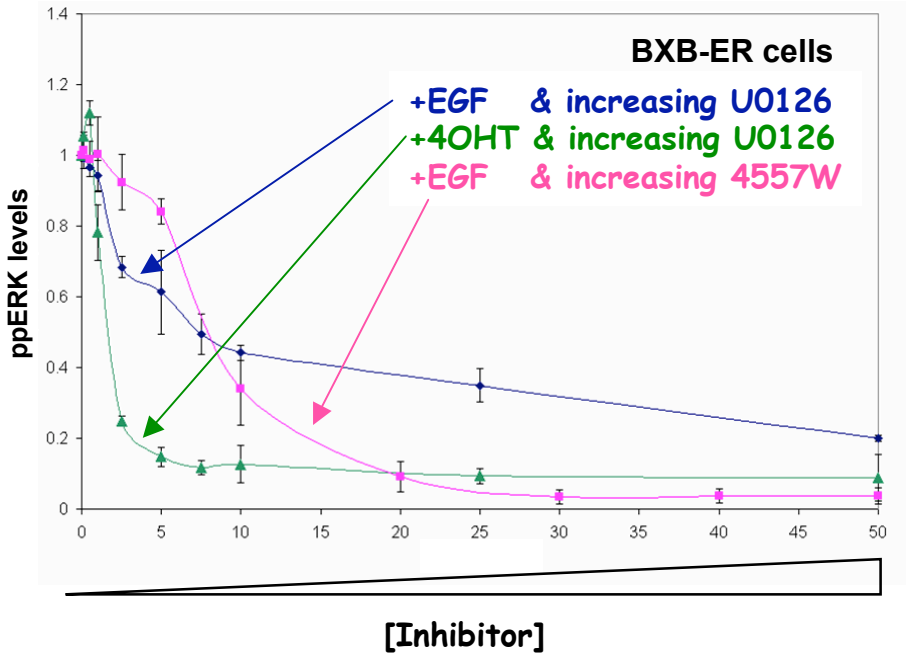
Comparison of EGFR vs MEK inhibition

MODEL



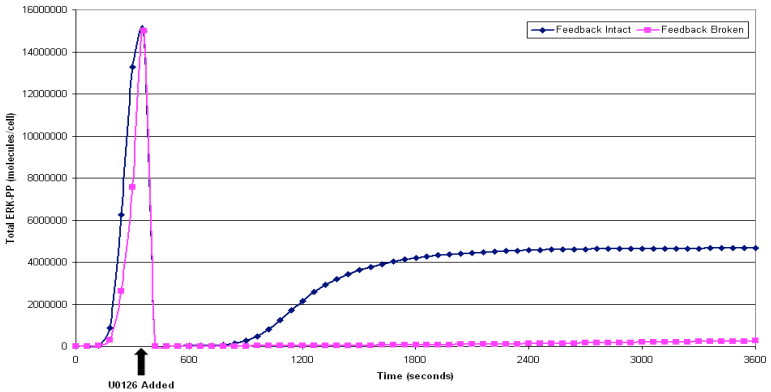
Almost linear decrease
in ERK-PP levels
i.e. less resistance to a EGFR inibitor

LAB

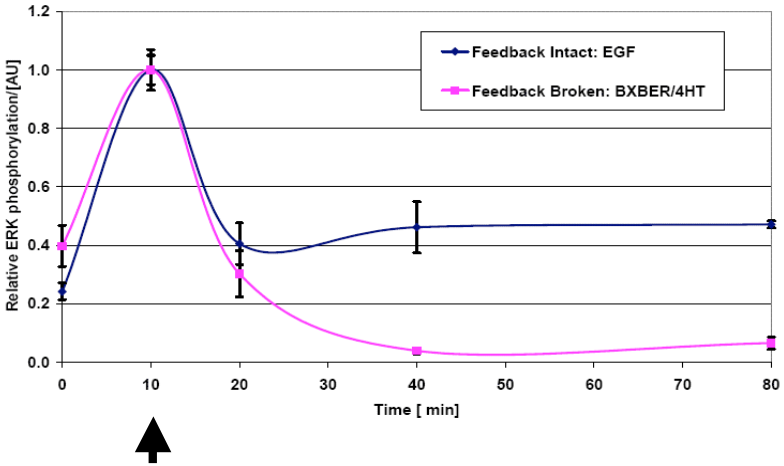


Signal recovery after MEK inhibition

Simulation



Experiment



U0126 added

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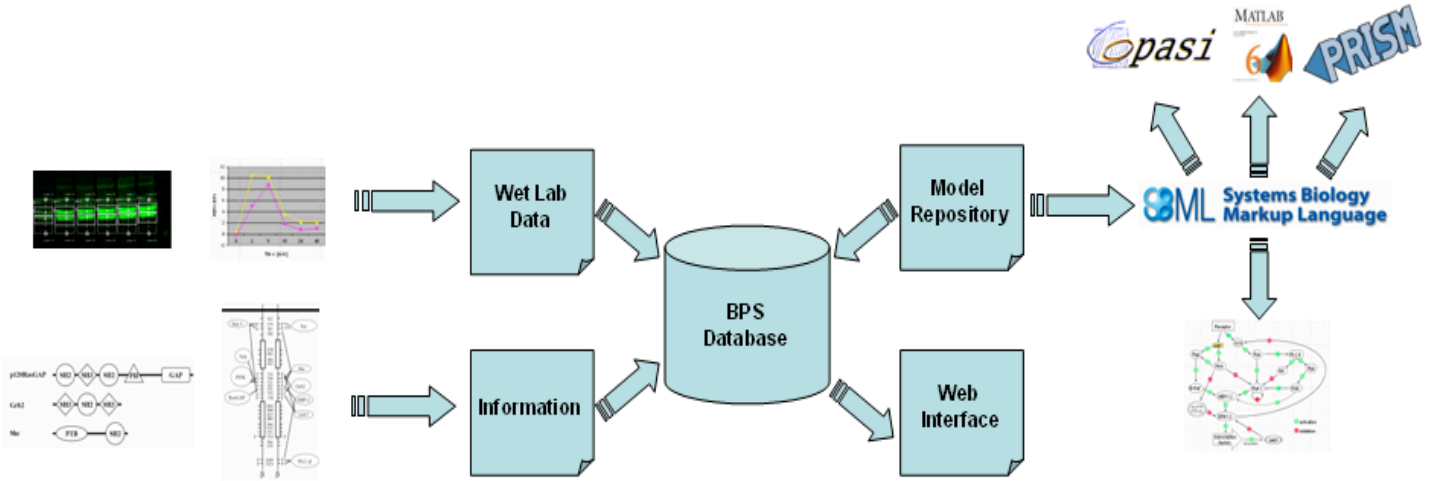
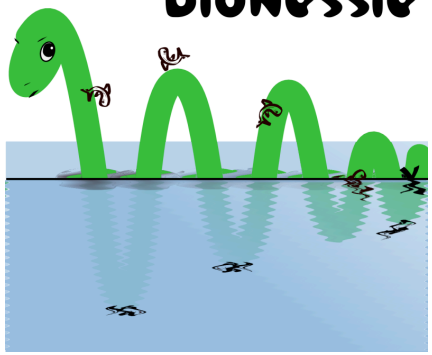
BPS Database

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

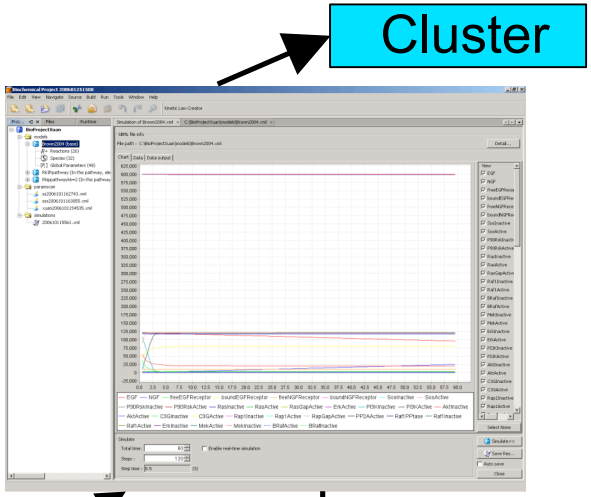


- We are also developing a number of software tools to aid in the modelling process:
  - Database import/export
  - Model visualisation
  - Model conversion



Simulator, analyser ...& *go-faster* on the Grid!

Xuan Liu, Vladislav Vyshemirsky, Gary Gray, Jipu Jiang, Femi Ajayi (David Gilbert, Richard Sinnott)

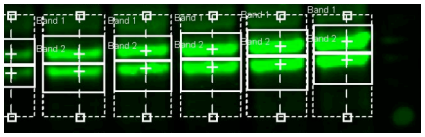


National e-Science Centre

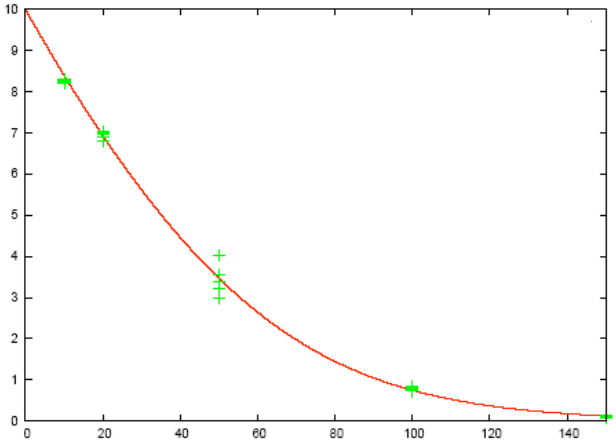
Using Bayesian Inference to model parameters

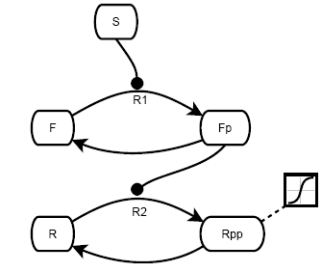
Vladislav Vyshemirsky & Mark Girolami

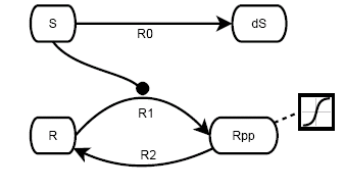
Lab data

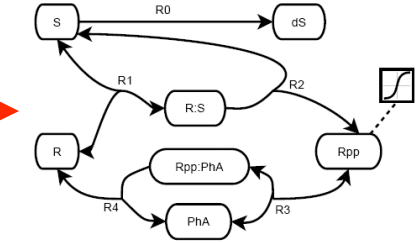


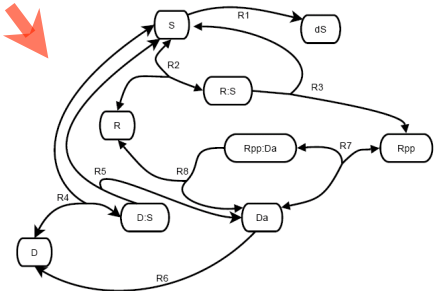
Parameter distributions











Model inference
Rate parameter inference
&
Topology comparison

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.
- Proteins embedded in negative feedback amplifier loops are difficult drug targets, as the system autocorrects inhibition
- Mathematical modelling can suggest where to interfere

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DTI Beacon Project Biological Pathway Simulator and Analyser

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

Joan Grindlay *Beatson Institute, Glasgow, UK*

UNIVERSITY of GLASGOW

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