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

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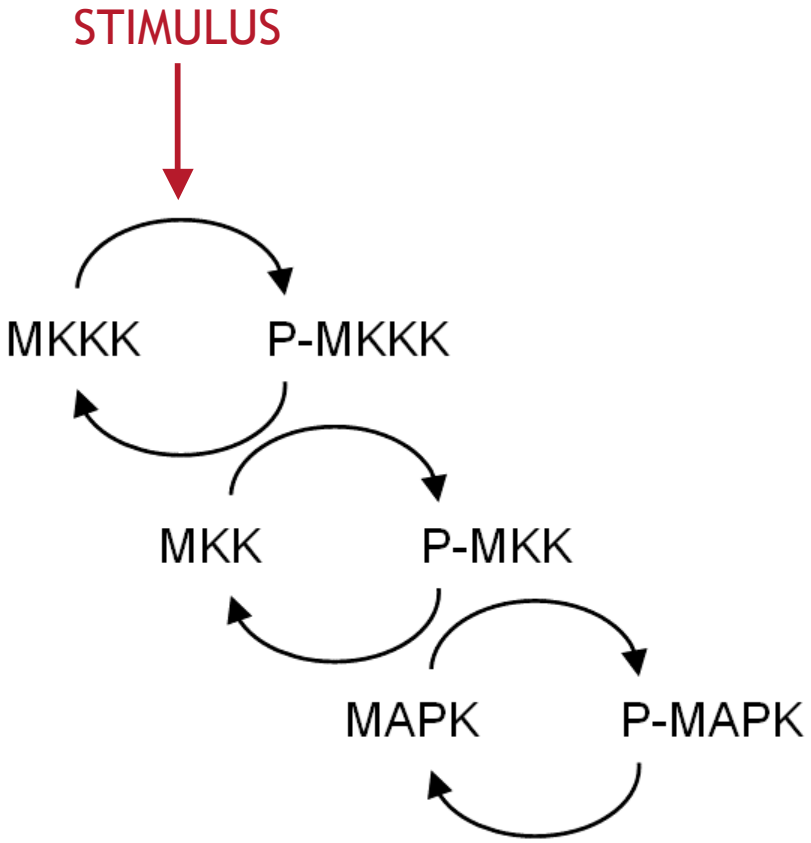
Structure of the talk

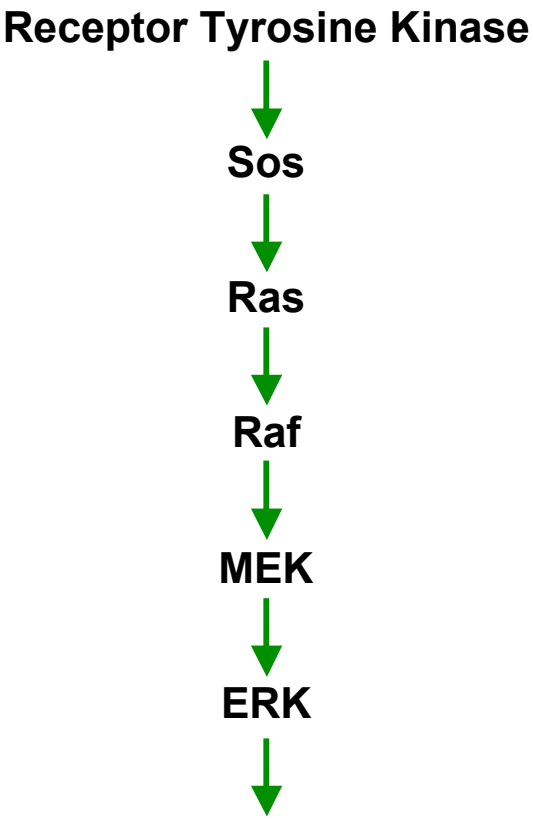
- MAPK cascade
- Feedback loops, theory ….
- Implications for drug targeting
- ODE model & simulations
- Experimental work and results
- Conclusions and outlook

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.

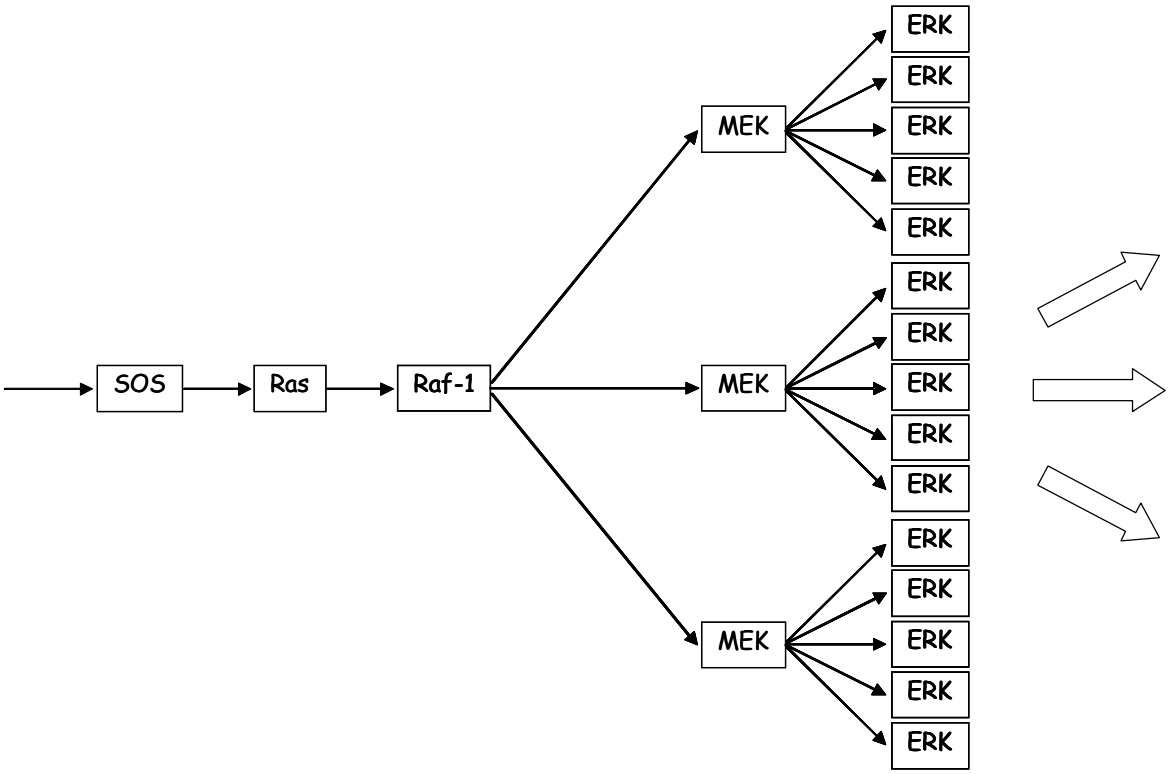


ERK Cascade



Amplification

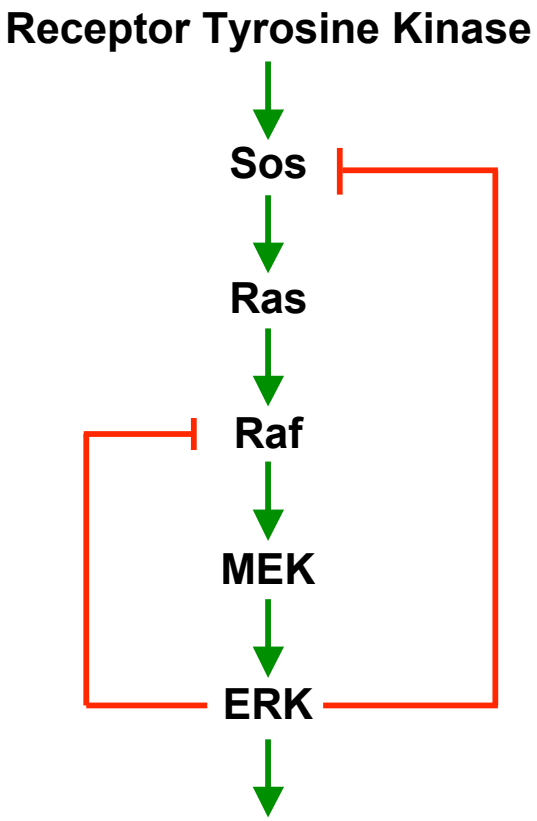
- ERK cascade well known biological amplifier -- amplifies the original signal to create effective cellular responses.
- 1:3:5 are the approximate ratios of Raf-1, MEK and ERK in fibroblasts.



Negative Feedback

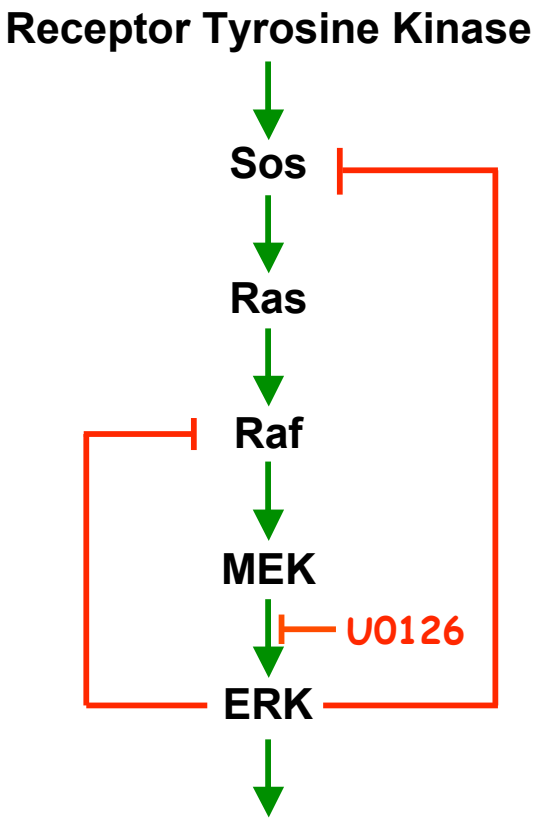
- Well known negative feedback loop: phosphorylation of SOS by ERK-PP (via MAPKAP1) resulting in the dissociation of the Grb2/SOS complex.

- 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

Current Drugs

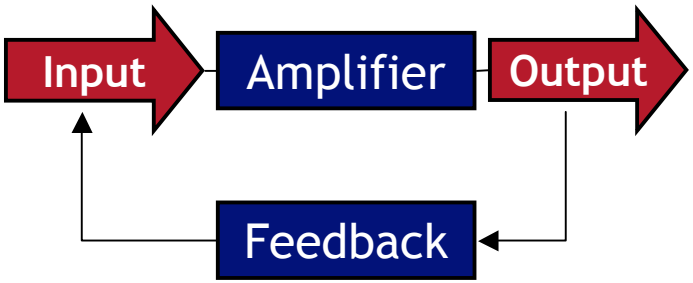


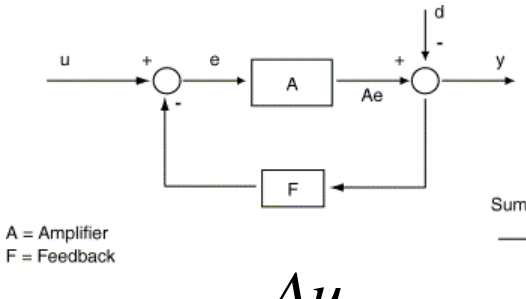
- Current drugs aimed at decreasing the activity of the MAPK pathway have proved less efficient in *in vivo* applications than anticipated from *in vitro* inhibition assays.
- E.g. U0126 inhibits MEK from phosphorylating and therefore activating ERK.
- One explanation for the poor performance of these drugs could be that the ERK cascade is a negative feedback amplifier.

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.

Sauro & Kholodenko (2004), Quantitative analysis of signaling networks, Progress in Biophysics & Molecular Biology 86(1) 5-43





Sauro & Kholodenko (2004)

$$y = \frac{Au}{1 + AF}$$

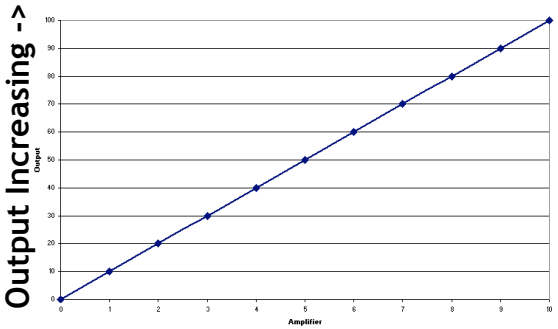
Steady State Equation

y = output; u = input; A = amplifier; F = feedback

Characteristics

- The negative feedback amplifier is much more robust to disturbances when compared to a standard amplifier.

Standard Amplifier



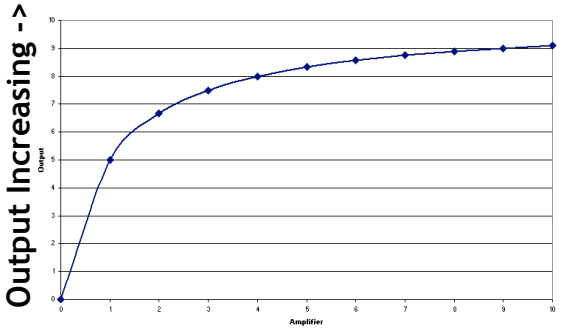
<- Amplifier Decreasing

y=Au

Linear Behaviour

As the amplifier is decreased there is a simple linear decrease in the system output

Feedback Amplifier



<- Amplifier Decreasing

y=Au/(1+AF)

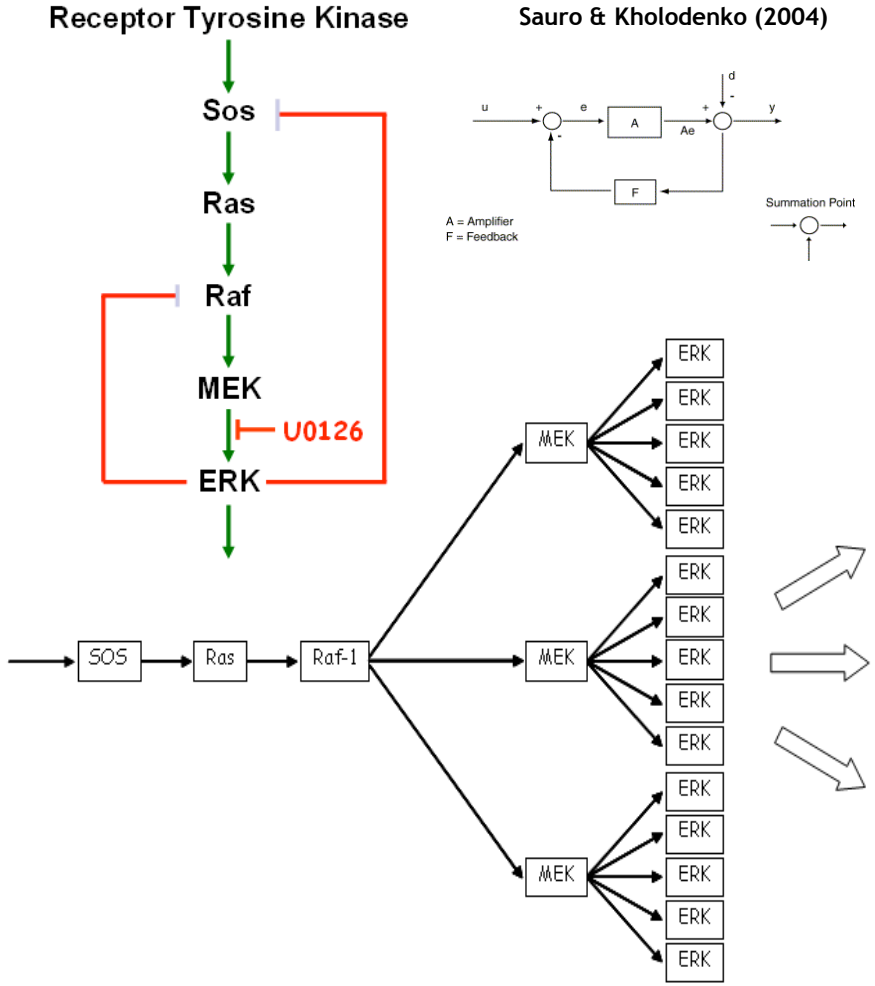
Robust Behaviour

As the amplifier is decreased the system 'resists' the disturbance

- However, in a feedback amplifier there is a significant loss in the gain of the amplifier.

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.

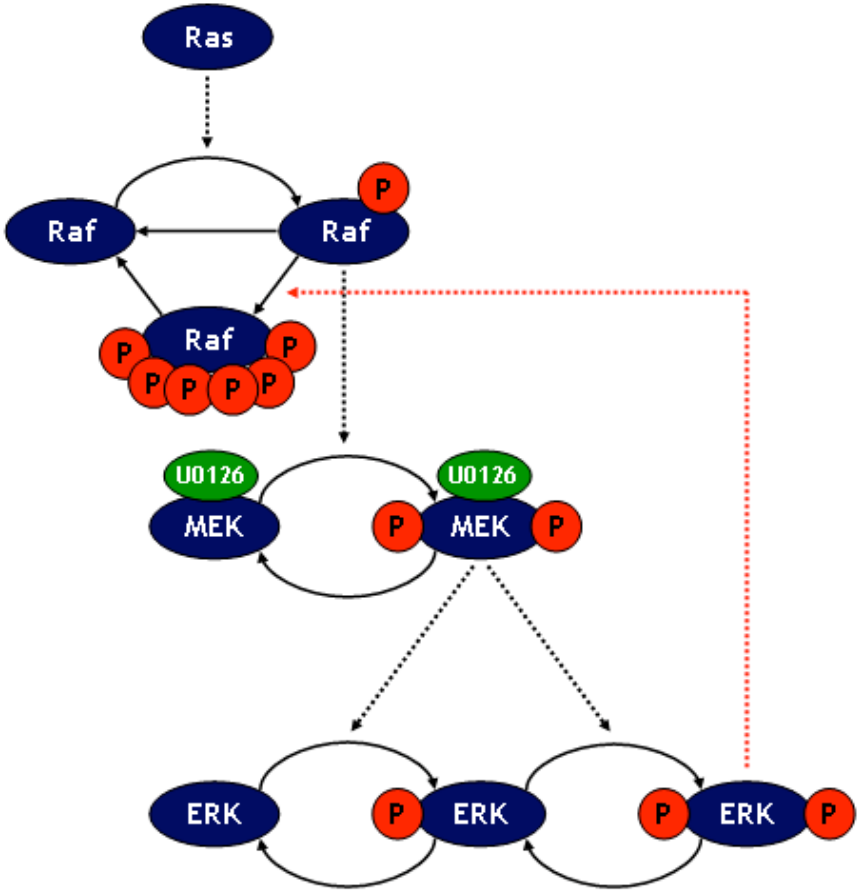


Experimental Strategy

- Developed a number of computational (ODE) models to investigate the feedback amplifier characteristics of the ERK cascade.
- Designed and performed a number of laboratory experiments to test whether the ERK cascade is indeed a feedback amplifier.
- The basic strategy is to monitor ERK-PP (output) levels with increasing concentrations of the MEK inhibitor U0126 (increasing disturbance) when the feedback loop is present and when it has been knocked out.

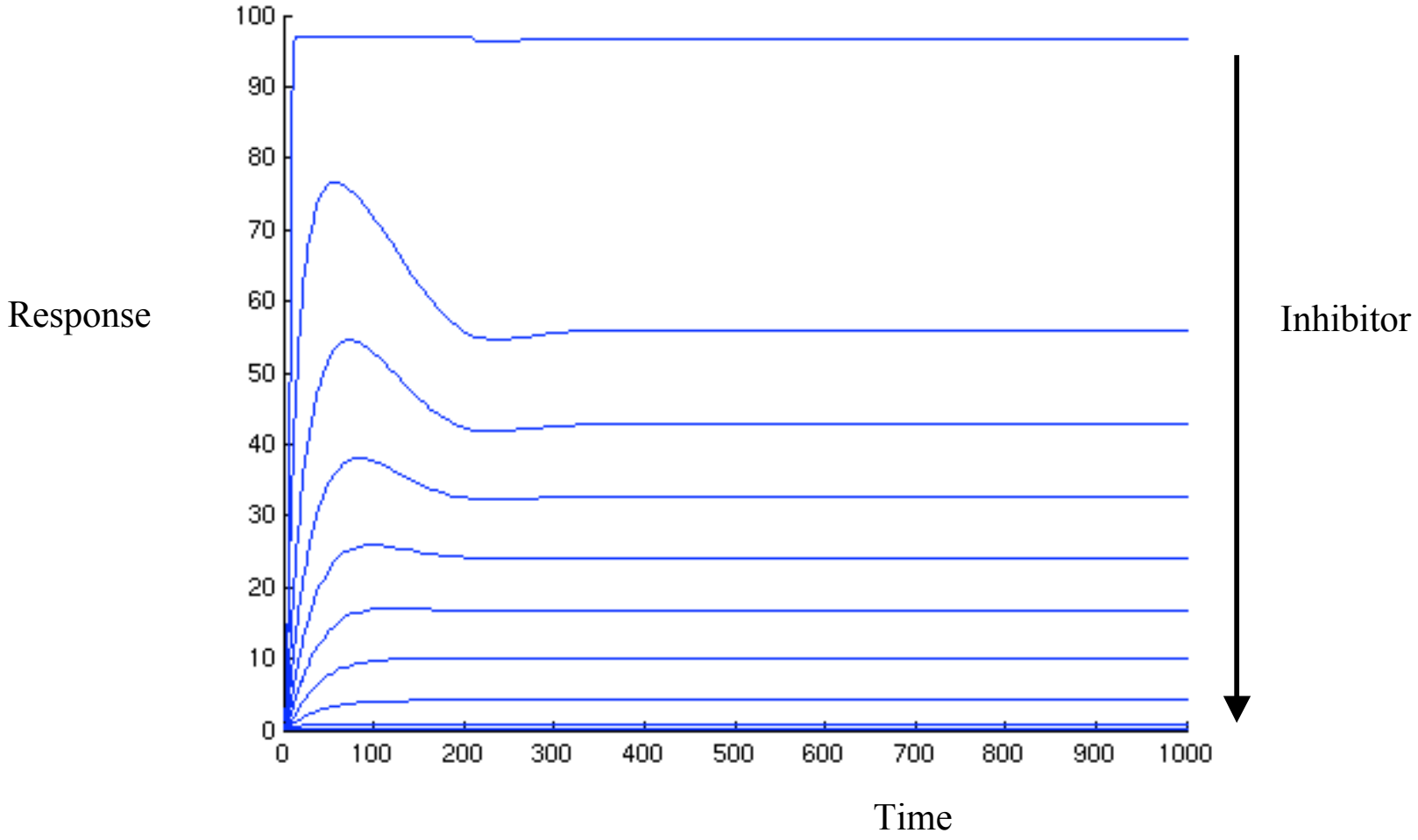
Computational Modelling 1

- Ras - RasV12, an always active form of Ras which gives a constant stimulus and therefore readily leads to a steady state in the system.
- Activation of MEK is represented as a single step as single phosphorylated MEK species have not been observed.
- Concentration of the MEK inhibitor U0126 is increased incrementally and steady state ERK-PP levels monitored.
- Negative feedback loop - hyper-phosphorylation of Raf by ERK-PP.
- Simulations run with and without the negative feedback loop present.
- Model is based on Brightman & Fell (2000)



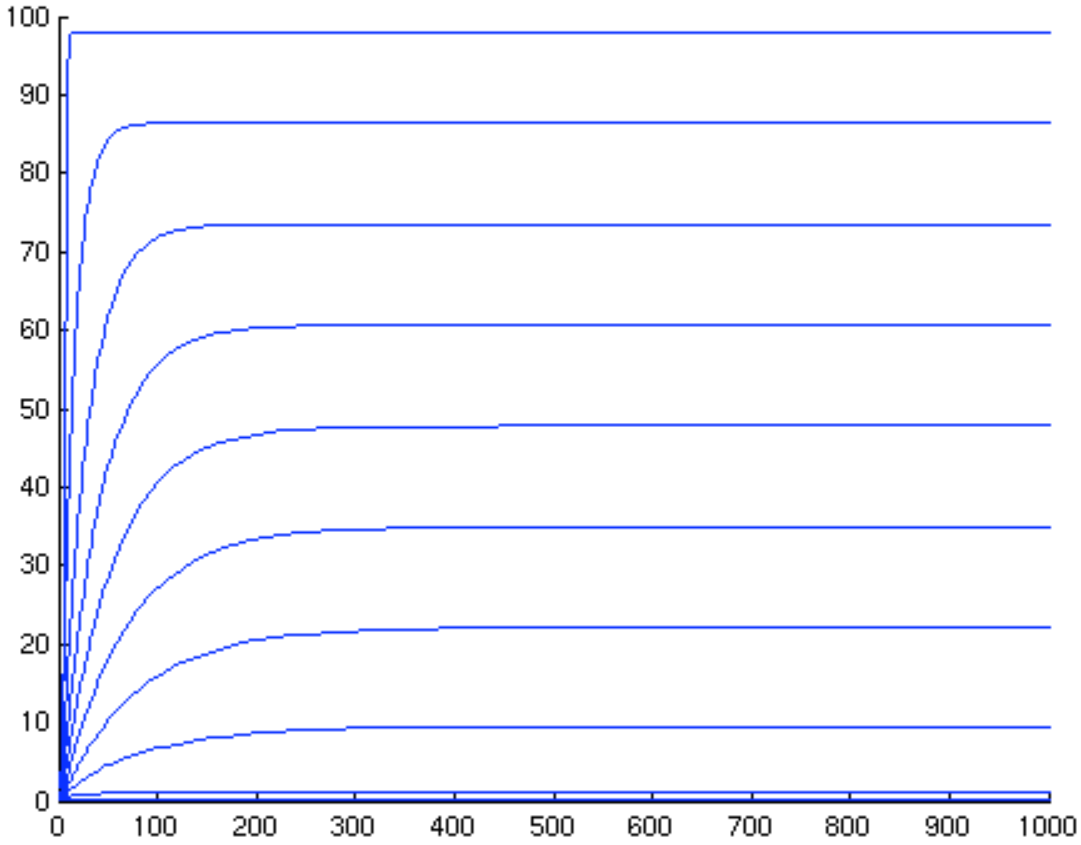
Brightman & Fell (2000), Differential feedback regulation of the MAPK cascade underlies the quantitative differences in EGF and NGF signalling in PC12 cells, FEBS Letters 482, 169-174

With feedback



No feedback

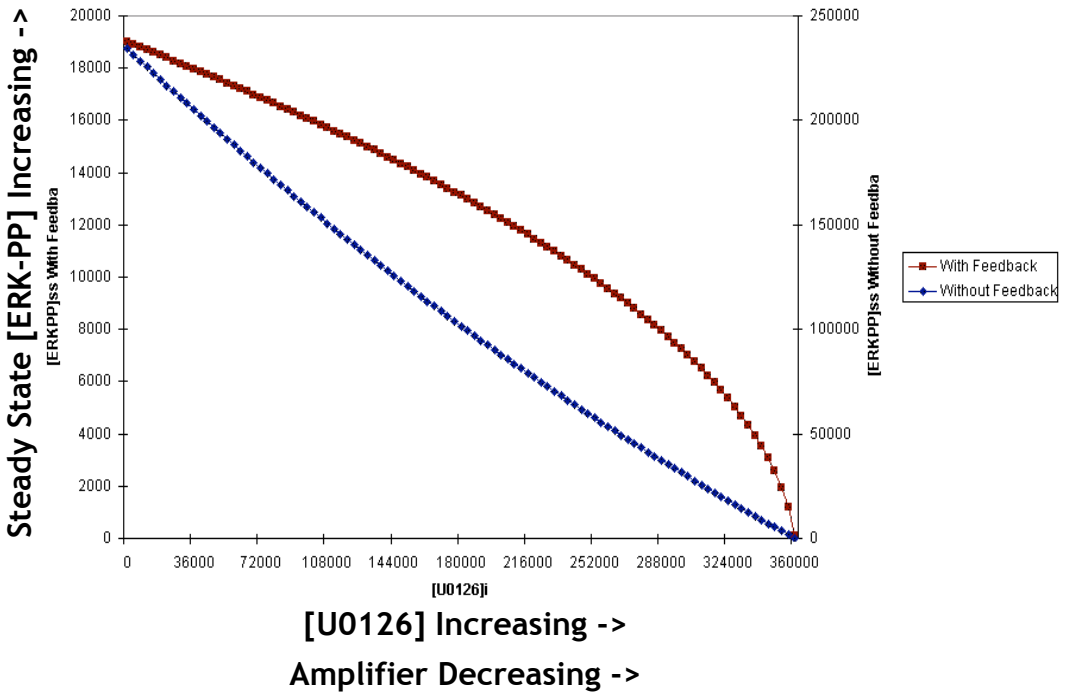
Response



Inhibitor

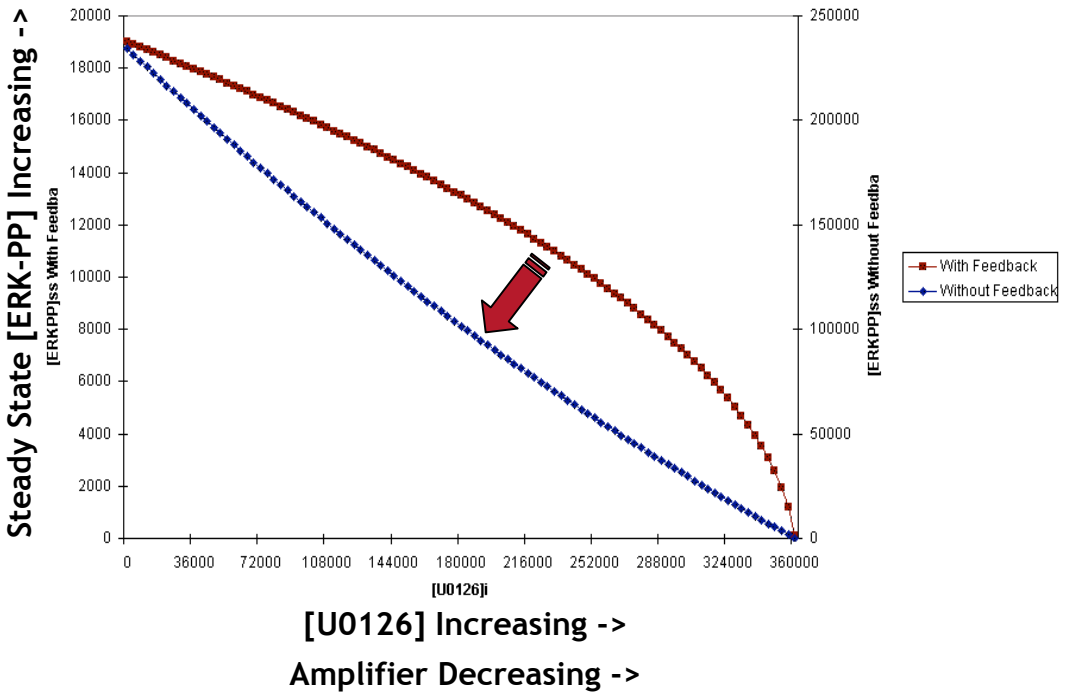
Simulation Results 1

- Increasing the concentration of U0126 essentially has the affect of decreasing the amplifier.
- Without the negative feedback loop ERK-PP levels decrease linearly.
- With the negative feedback loop ERK-PP levels are much more resistant to U0126.
- This conforms relatively well with negative feedback amplifier system behaviour.



Varying Feedback Strength

- Decreasing the strength of the negative feedback loop (via the kinetic constants of the reactions) decreasing the robustness of the system.
- Therefore, the stronger the feedback loop the more robust the system.
- Again, this conforms relatively well with negative feedback amplifier system behaviour.

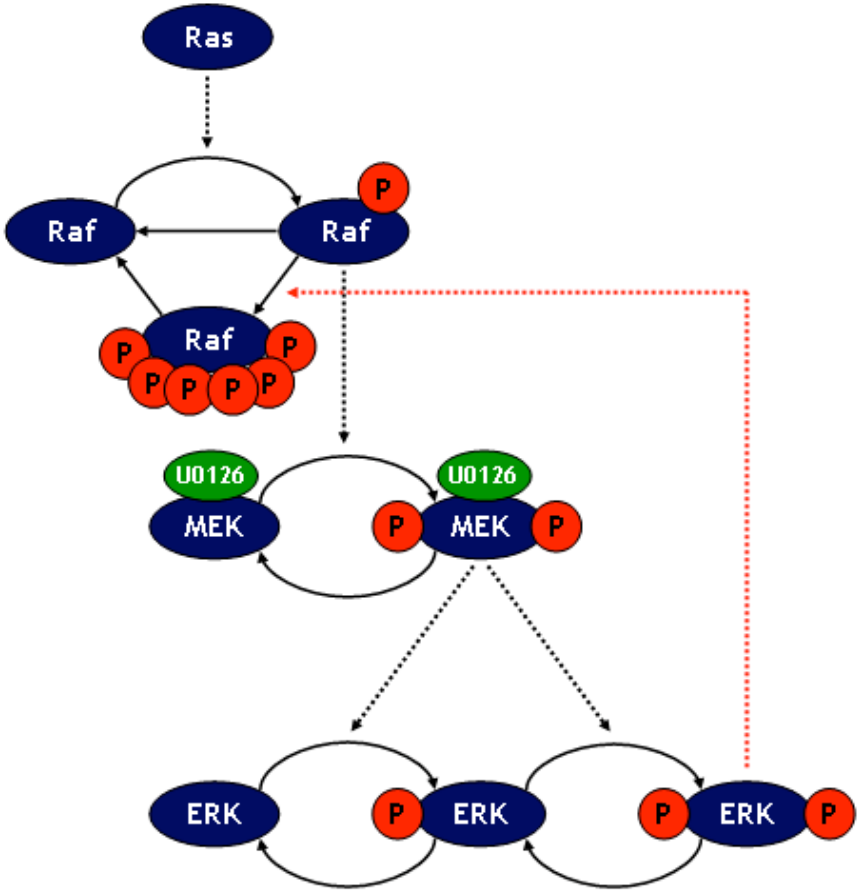


Michaelis-Menten

- ERK-PP levels monitored at the steady state.
- However, the Brightman & Fell (2000) model contains numerous reactions that utilise (standard) Michaelis-Menten type kinetics.
- The Michaelis-Menten equation makes a number of assumptions which may make it unsuitable for steady state analyses:
  - No product inhibition:
  - No product conversion back to substrate (however small)
  - [S] is much greater than [E]
- Therefore, another model of the ERK cascade was developed using pure mass actions kinetics.

Computational Modelling 2

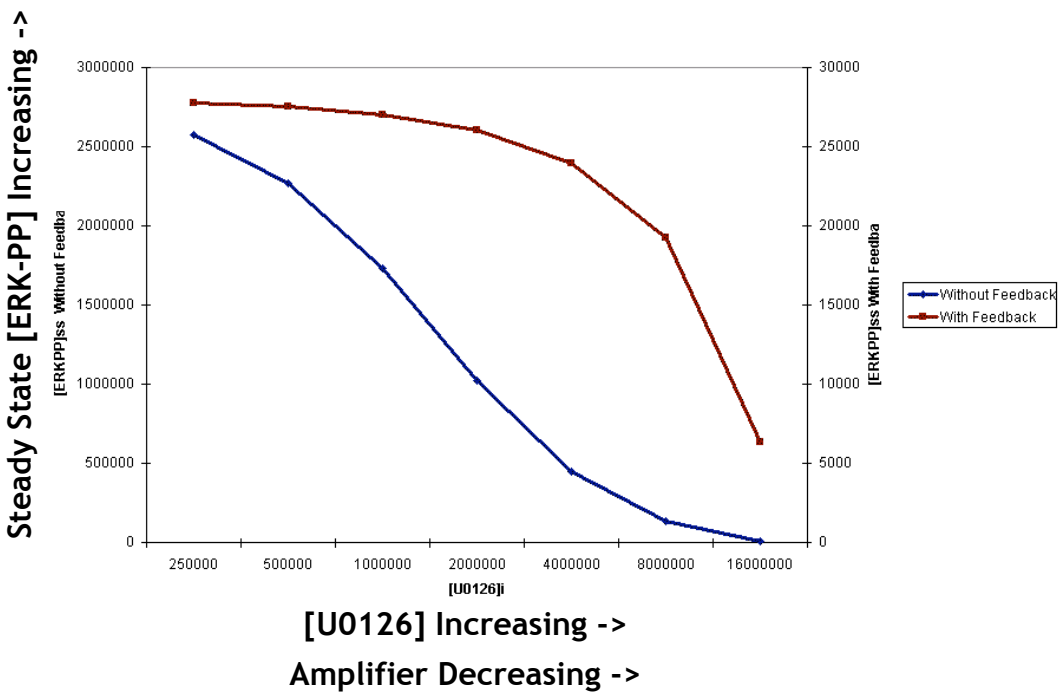
- Pure mass action model of the ERK cascade
- Same topological structure as the michaelis-menten model.
- Model based on Schoeberl *et al*. (2000)



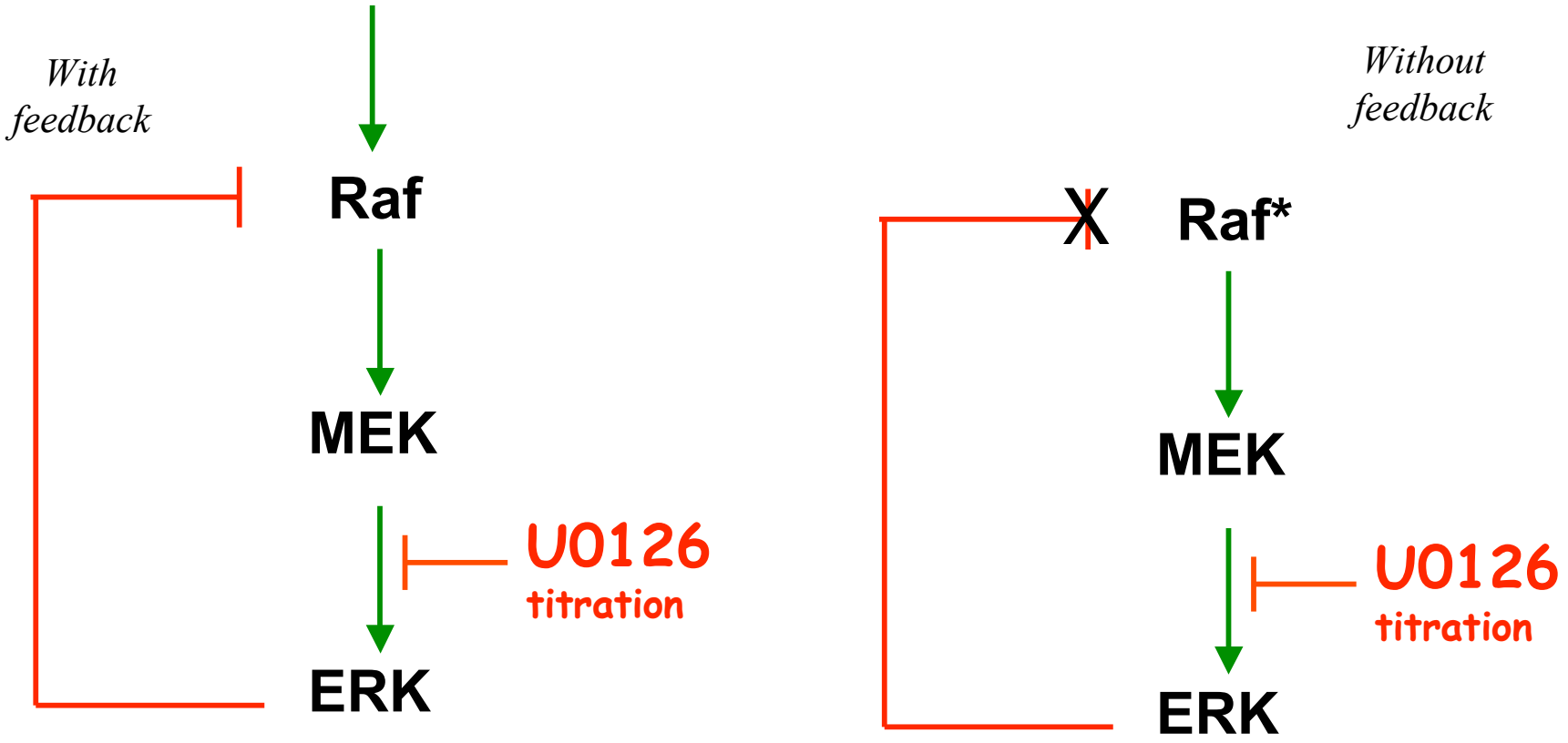
Schoeberl *et al*. (2002), Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors, Nature Biotechnology 20, 370-375

Simulation Results 2

- Once again, there are clear differences in system behaviour with and without the negative feedback loop.
- Without the negative feedback loop ERK-PP levels decrease linearly .
- With the negative feedback loop ERK-PP levels are much more resistant to U0126.
- Like the michaelis-menten model, this pure mass action also conforms relatively well with negative feedback amplifier system behaviour.

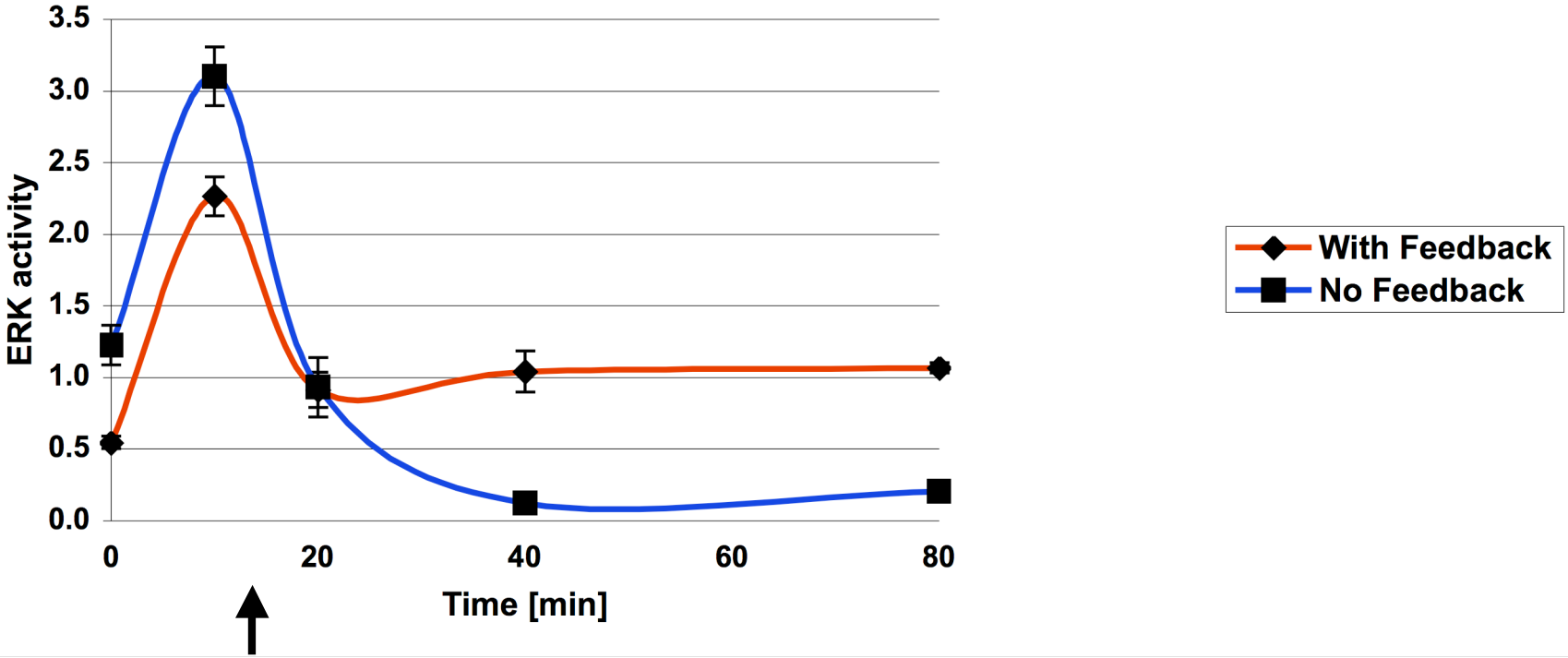


Experimental interventions

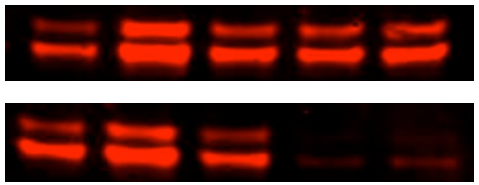


- U0126 inhibitor
- Breaking the feedback loop ….

Signal recovery of ERK after adding MEK inhibitor
10 min after stimulation with and without feedback



U0126 added

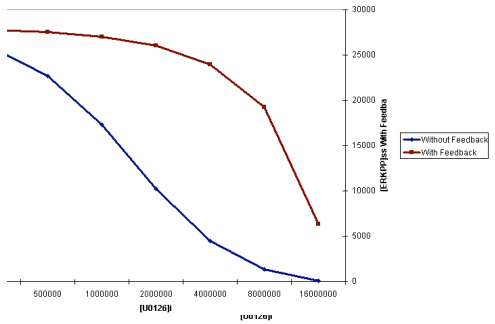


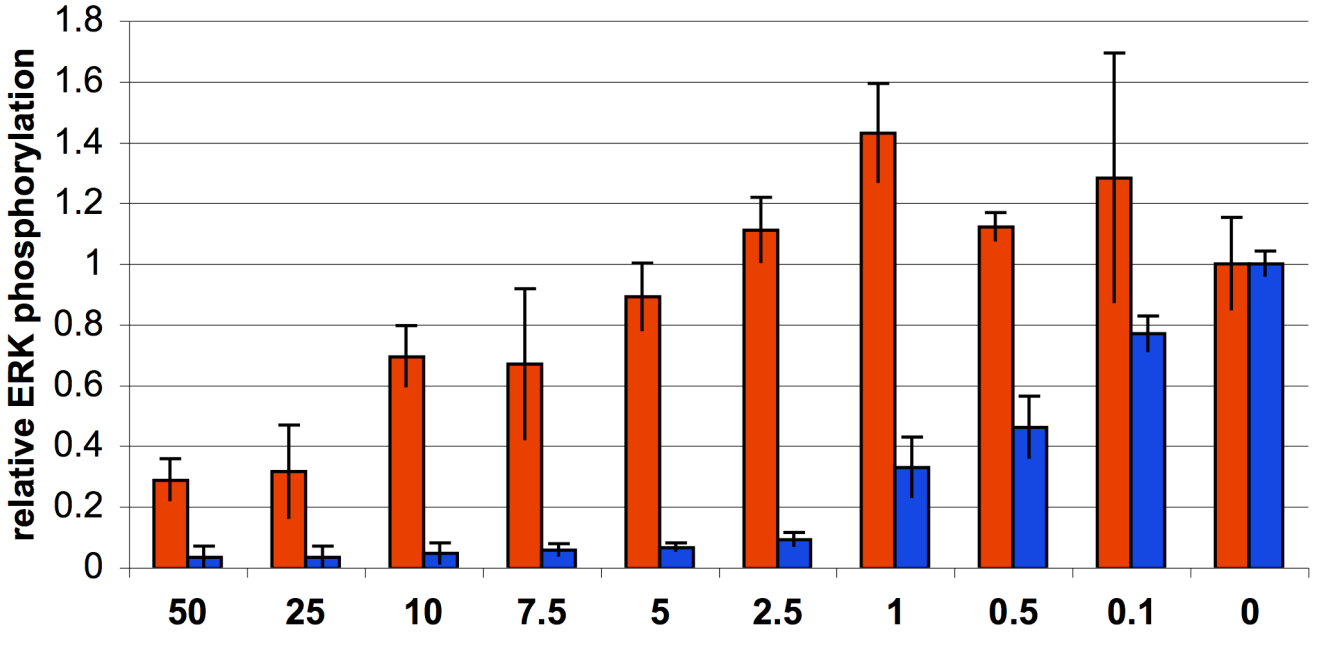
pERK, with Feedback, EGF stimulation

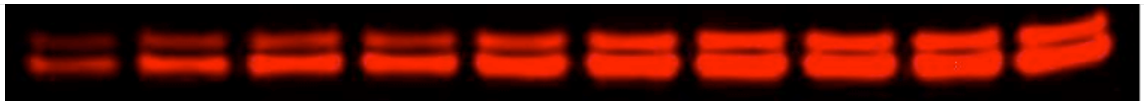
pERK, without Feedback, Raf construct

0 10 20 40 80 min stimulation

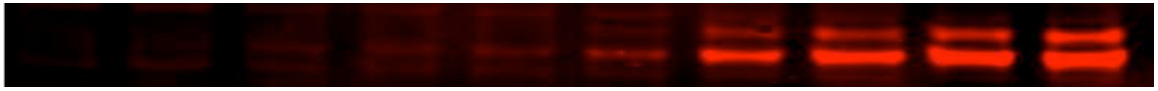
Effect of feedback amplification on sensitivity to signal disruption in the Raf/MEK/ERK







pERK, with feedback



pERK, no feedback

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

BPS: Biochemical Pathway Simulator
A Software Tool for Simulation & Analysis of Biochemical Networks

www.brc.dcs.gla.ac.uk/projects/bps

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Department of Trade and Industry 'Beacon' project

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