Brunel UNIVERSITY LONDON

Modelling and analysing the effects of feedback in signal transduction pathways

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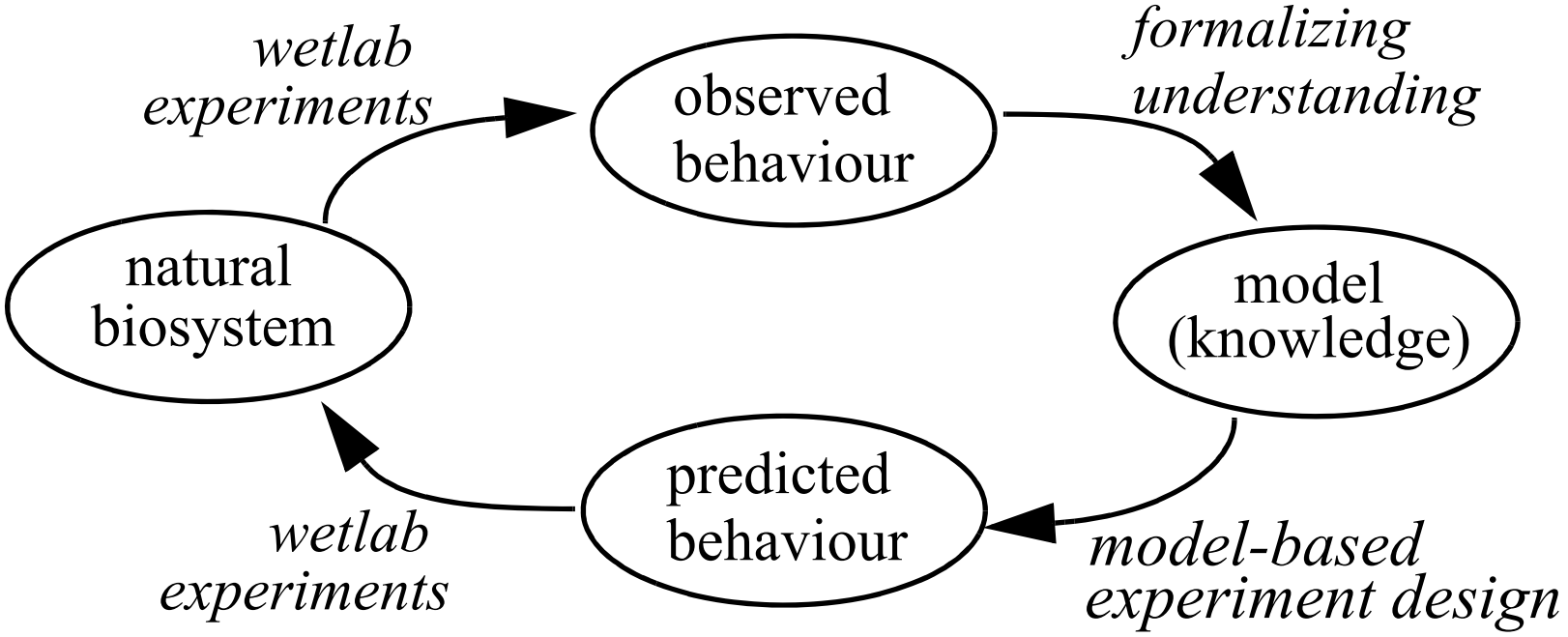
Subtext

- Can modelling ever be useful?
  - For Pharmacology???
- What did a modelling activity tell us?
- (When) should we invest in modelling?

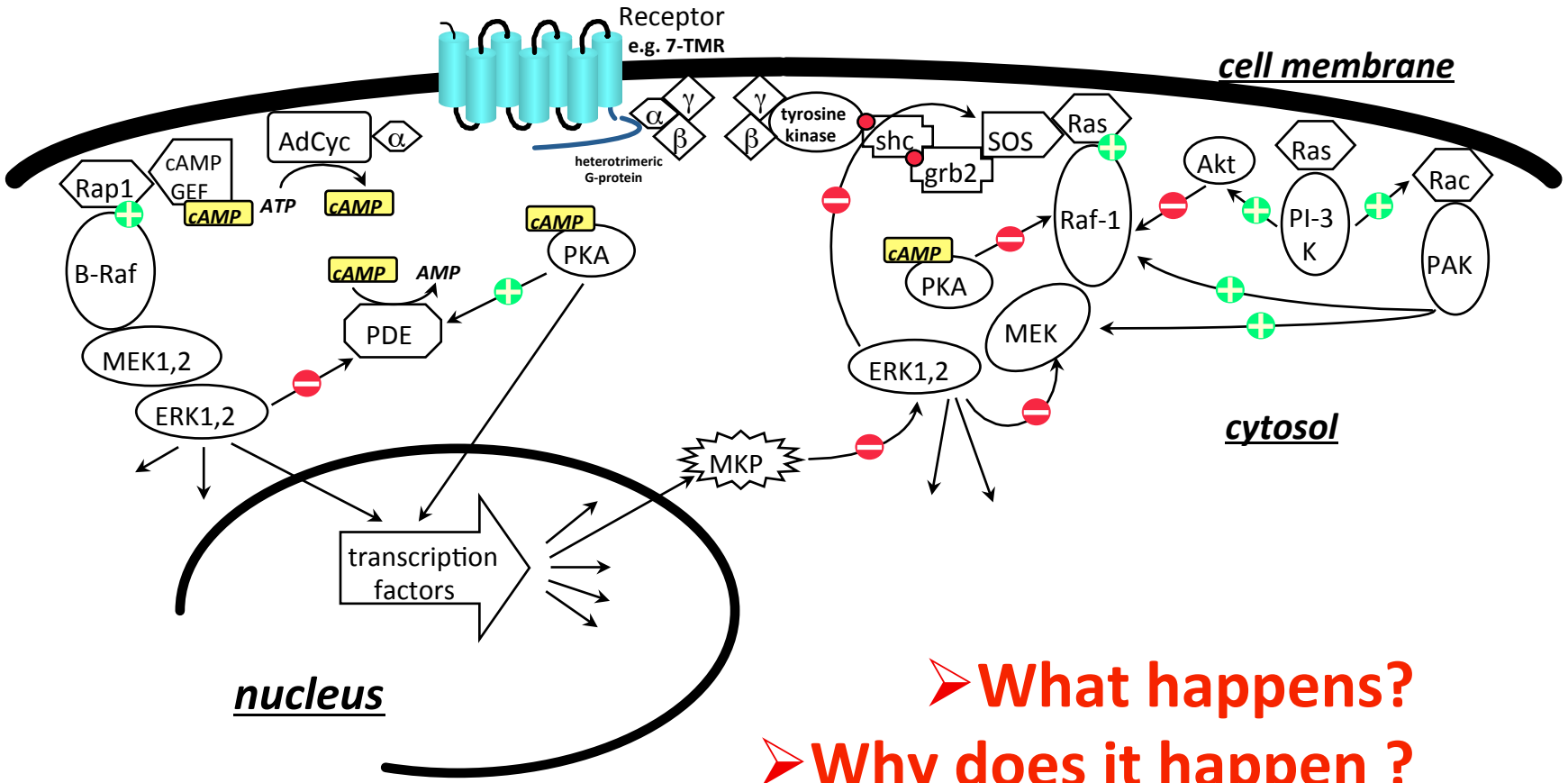
Systems Biology

systems biology: modelling as formal knowledge representation

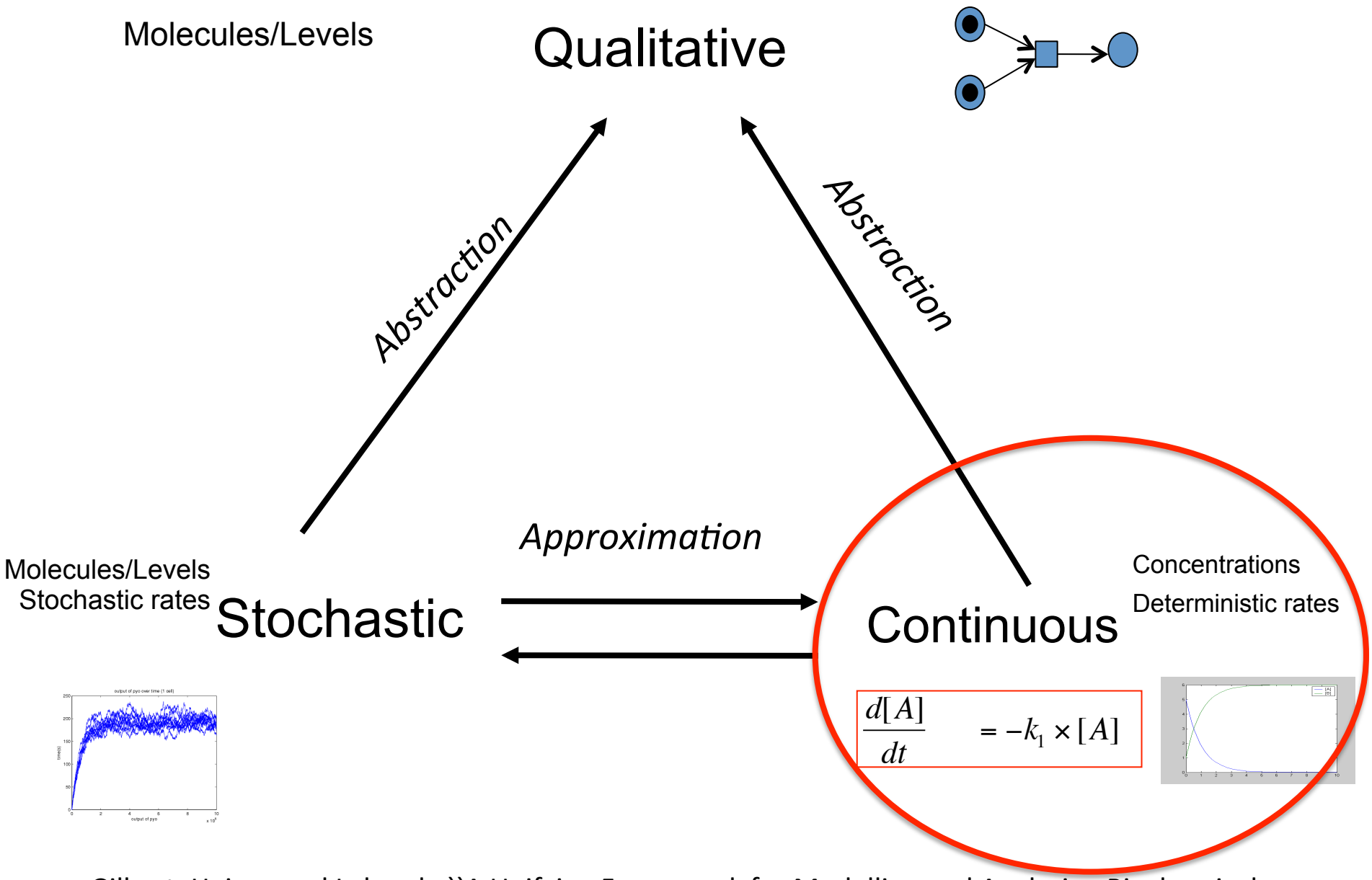


Biochemical networks

We can describe the general topology and single biochemical steps. However, we do not understand the network function as a whole.



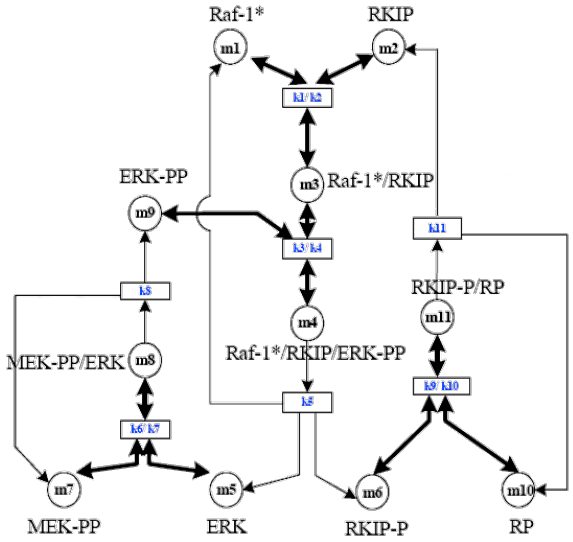
- **What happens?**
- **Why does it happen ?**
- **How is specificity achieved?**



Gilbert, Heiner and Lehrack. ``A Unifying Framework for Modelling and Analysing Biochemical Pathways Using Petri Nets.” Proc CMSB 2007

What is a biochemical network model?

1. Structure



graph
QUALITATIVE

2. Kinetics (if you can)

reaction rates

$$d[Raf1^*]/dt = k1*m1*m2 + k2*m3 + k5*m4$$

$$k1 = 0.53;\ k2 = 0.0072;\ k5 = 0.0315$$

QUANTITATIVE

3. Initial conditions

marking , concentrations

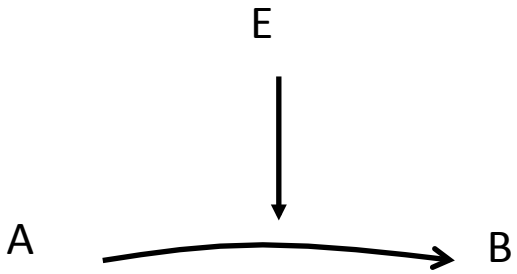
$[Raf1^*]_{t=0}$= 2 μMolar

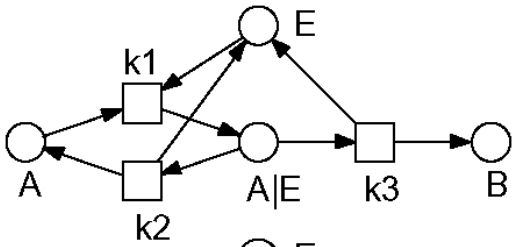
QUANTITATIVE

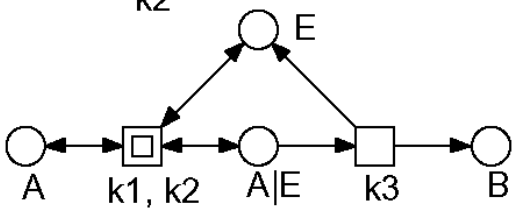
Mass action for enzymatic reaction - phosphorylation

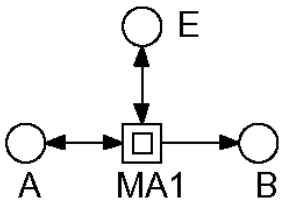
$$E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E \mid A \xrightarrow{k_3} E + B$$

- A: substrate
- B: product (phosphorylated A)
- E: enzyme (kinase)
- E|A substrate-enzyme complex









Breitling, Gilbert, Heiner & Orton "A structured approach for the engineering of biochemical models, illustrated for signalling pathways". Briefings in Bioinformatics, 2008

Differential equations

Enzymatic reaction

$$A + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} A \mid E \xrightarrow{k_3} B + E$$

$$\frac{d[A]}{dt} = -k_1 \times [A] \times [E] + k_2 \times [A \mid E]$$

$$\frac{d[A \mid E]}{dt} = +k_1 \times [A] \times [E] - k_2 \times [A \mid E] - k_3 \times [A \mid E]$$

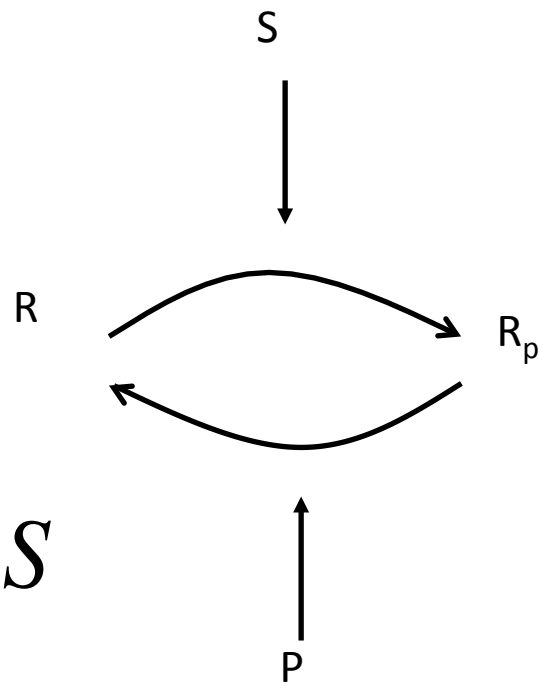
$$\frac{d[B]}{dt} = + k_3 \times [A \mid E]$$

$$\frac{d[E]}{dt} = -k_1 \times [A] \times [E] + k_2 \times [A \mid E] + k_3 \times [A \mid E]$$

Breitling, Gilbert, Heiner & Orton "A structured approach for the engineering of biochemical models, illustrated for signalling pathways". Briefings in Bioinformatics, 2008

Phosphorylation - dephosphorylation step
Mass action

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

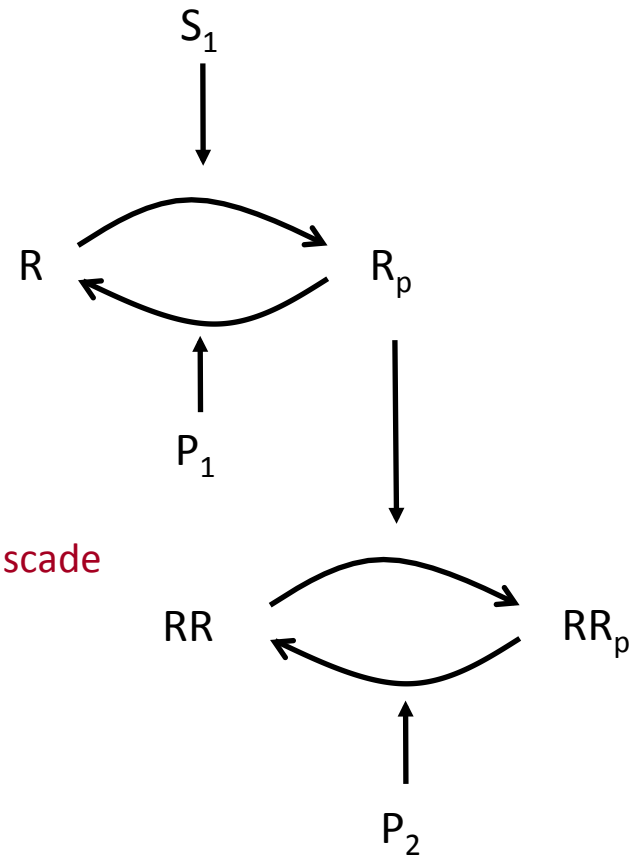


$$R+S \underset{k_2}{\overset{k_1}{\rightleftarrows}} R|S \xrightarrow{k_3} R_p + S$$

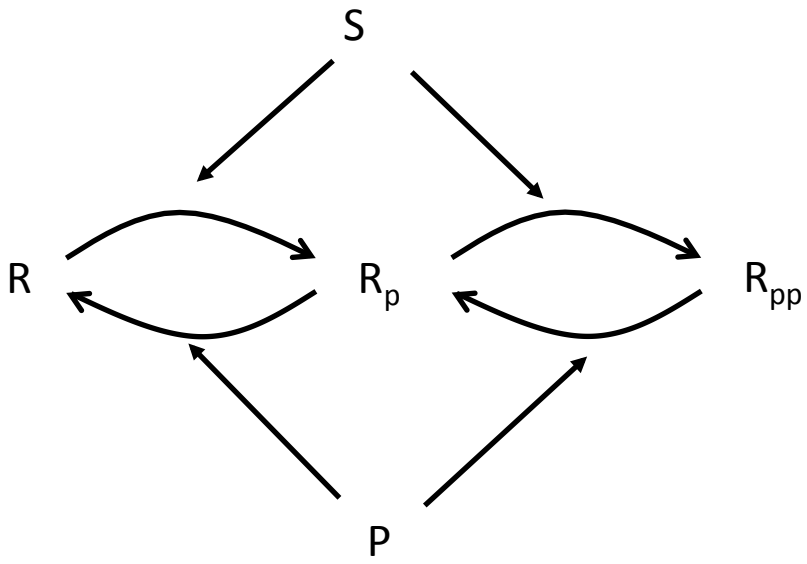
$$R+P \xleftarrow{kr_3} R_p|P \underset{kr_2}{\overset{kr_1}{\rightleftarrows}} R_p + P$$

Breitling, Gilbert, Heiner & Orton "A structured approach for the engineering of biochemical models, illustrated for signalling pathways". Briefings in Bioinformatics, 2008

Composition
Vertical & horizontal

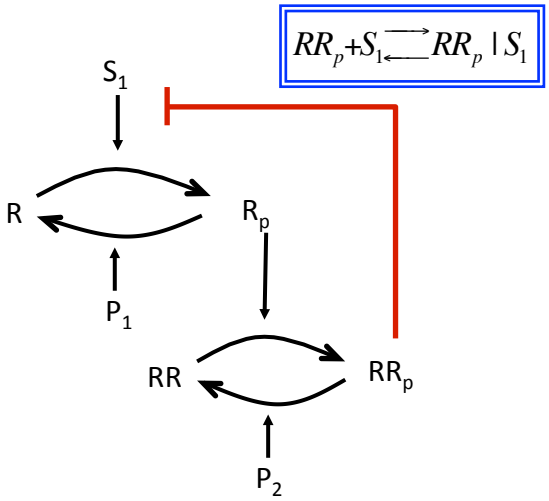


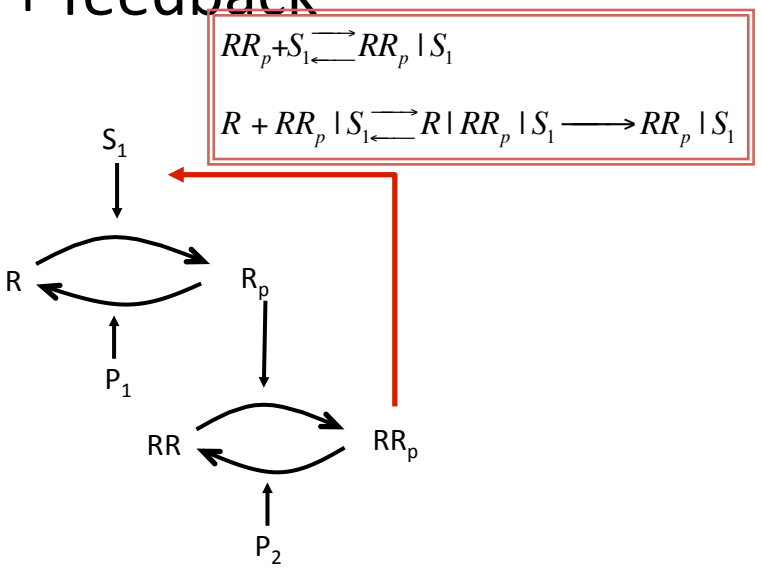
2-stage cascade

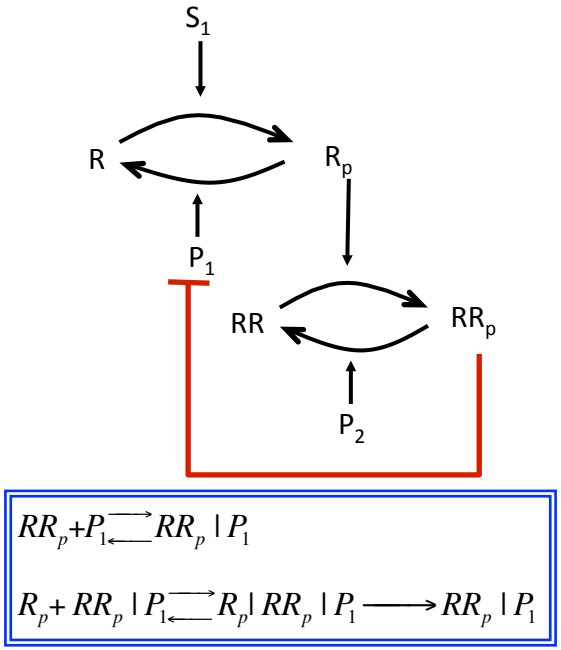


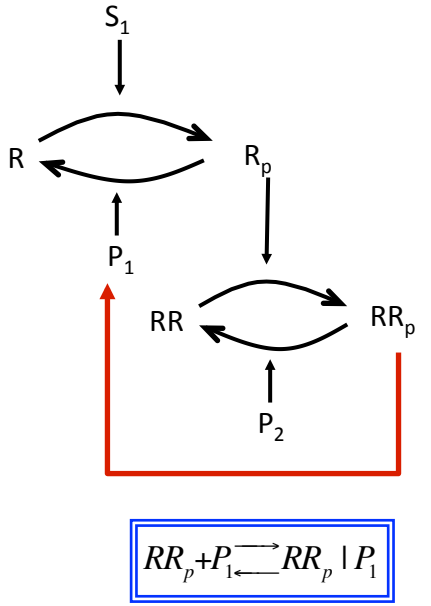
1-stage cascade
double phosphorylation step

Phosphorylation cascade + feedback









Phosphorylation cascade, negative feedback. Inhibitor on 2nd stage

$$RRR_p + S_1 \underset{ki'}{\overset{ki}{\rightleftarrows}} RRR_p \mid S_1$$

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

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

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

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

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

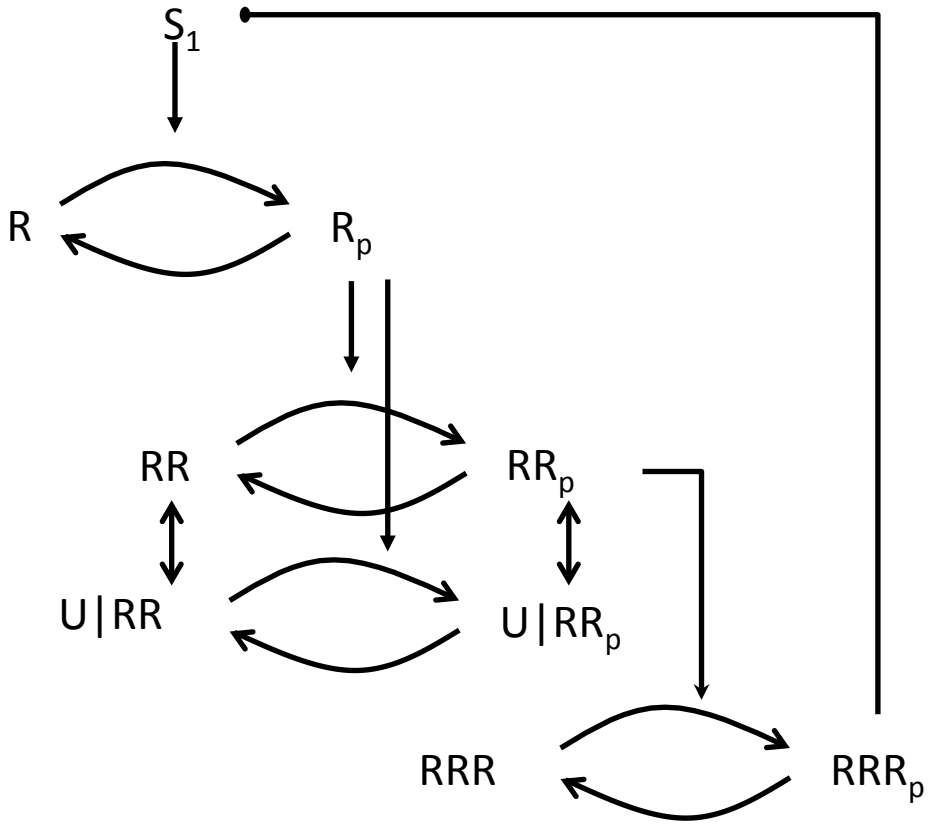
$$RRR + P_3 \xleftarrow{kkk_3'} RRR_p \mid P_3 \underset{kkk_2'}{\overset{kkk_1'}{\rightleftarrows}} RRR_p + P_3$$

$$U + RR \underset{ku_2}{\overset{ku_1}{\rightleftarrows}} U \mid RR$$

$$U + RR_p \underset{ku_2}{\overset{ku_1}{\rightleftarrows}} U \mid RR_p$$

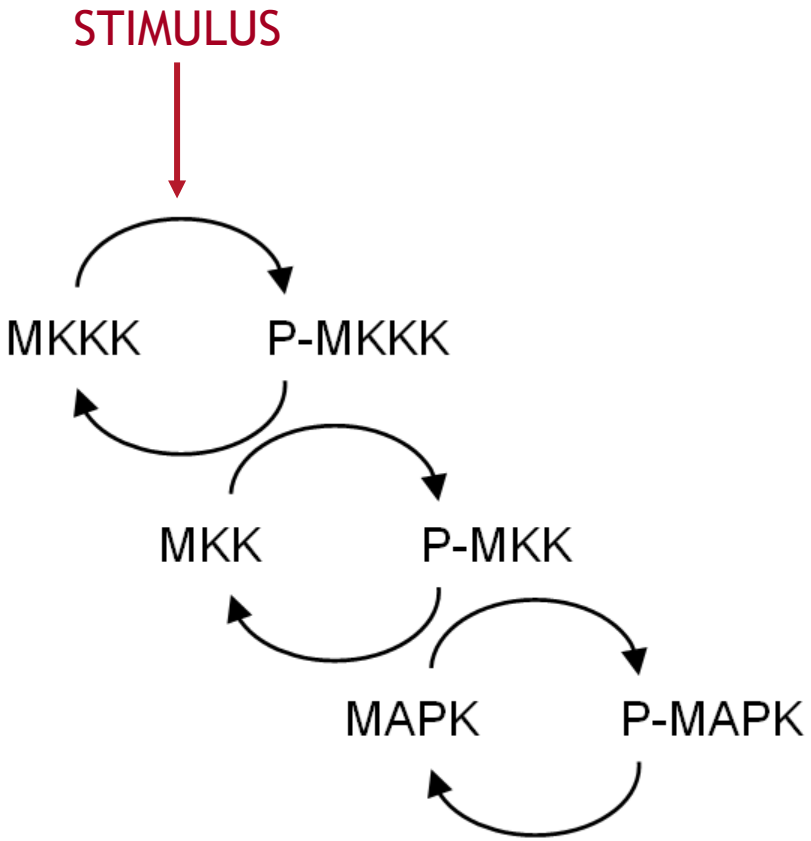
$$U \mid RR + R_p \underset{kk_2}{\overset{kk_1}{\rightleftarrows}} U \mid RR \mid R_p \xrightarrow{kk_3} U \mid RR_p + R_p$$

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



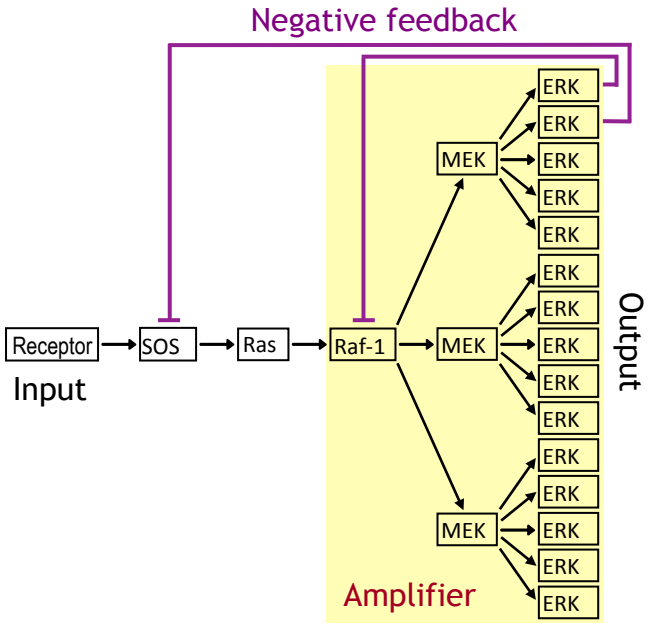
MAPK Pathway

- 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.



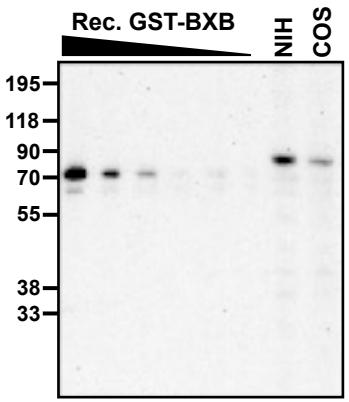
ERK cascade well known biological amplifier

- Amplifies the original signal to create effective cellular responses.
- 1:3:5 are the approximate ratios of Raf-1, MEK and ERK in fibroblasts.
- 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)

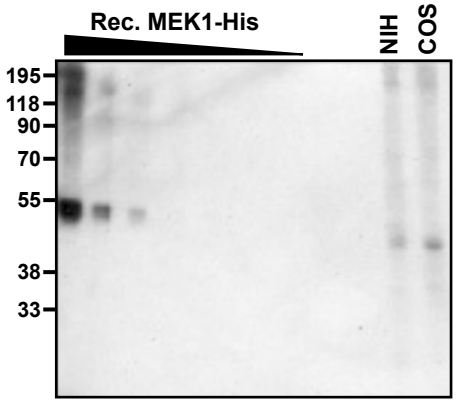


Dougherty *et al*. (2005), Regulation of Raf-1 by Direct Feedback Phosphorylation, Molecular Cell 17 215-224

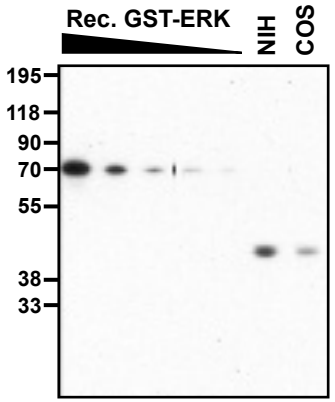
Raf/MEK/ERK amplifies the signal



WB: Raf-1



WB: MEK

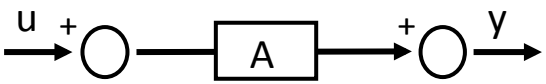


WB: ERK

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

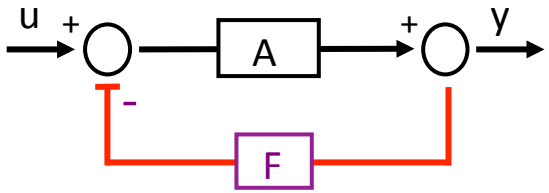
Negative Feedback Amplifier

Standard Amplifier



$y = u*A$

Negative Feedback Amplifier



$$y = u \frac{A}{1+A*F}$$

- Negative feedback amplifier from electronics
- Amplifier with a negative feedback loop from the output of the amplifier to its input.

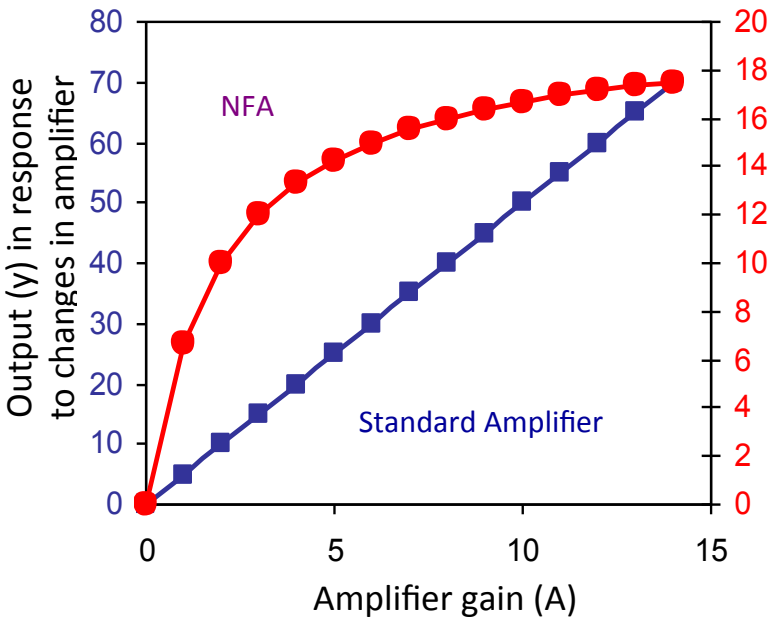
- NF loop → a system much more robust to disturbances in the amplifier.

- NFA was invented in 1927 by Harold Black of Western Electric.
- Originally used for reducing distortion in long distance telephone lines.

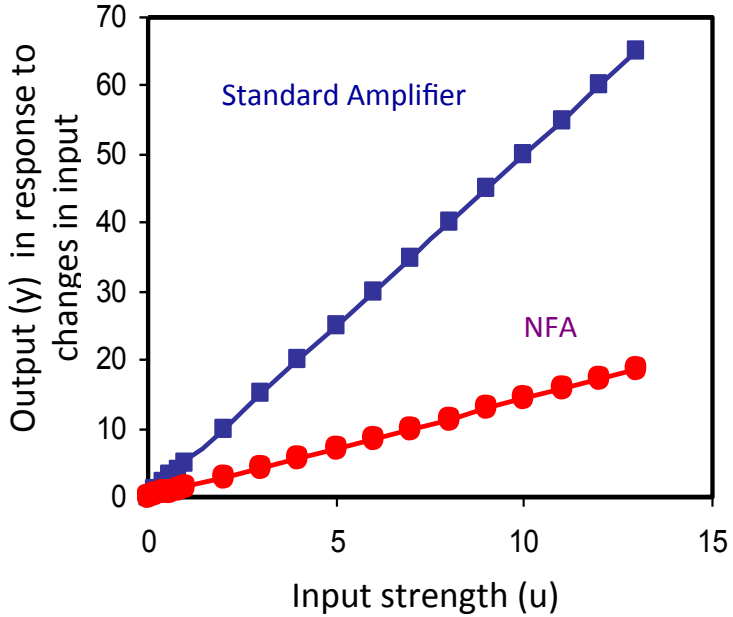
- NFA a key electrical component used in a wide variety of applications

Figure 1

C)

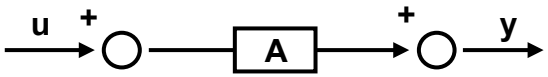

Output (y) in response to changes in amplifier
80
70
60
50
40
30
20
10
0
20
18
16
14
12
10
8
6
4
2
0
NFA
Standard Amplifier
0
5
10
15
Amplifier gain (A)

D)


Output (y) in response to changes in input
70
60
50
40
30
20
10
0
Standard Amplifier
NFA
0
5
10
15
Input strength (u)

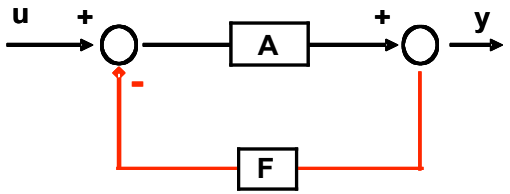
The negative feedback imparts signalling robustness

Standard Amplifier



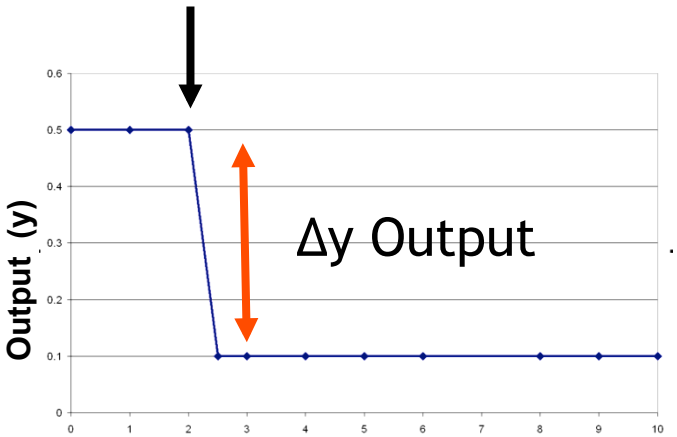
y=A*u

Negative Feedback Amplifier

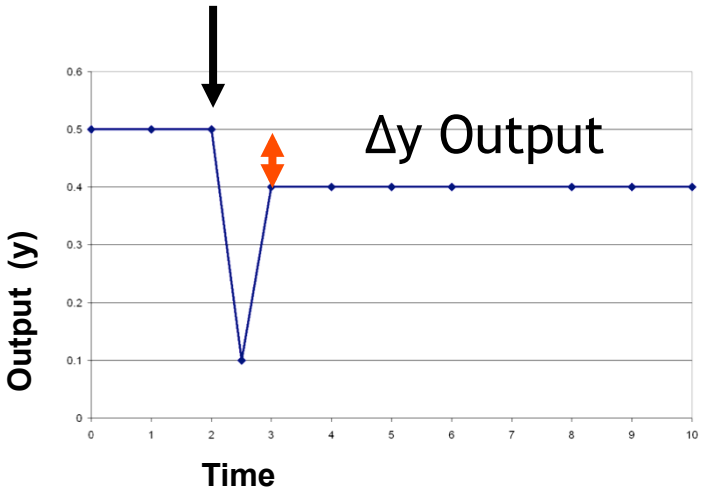


y=A*u/(1+A*F)

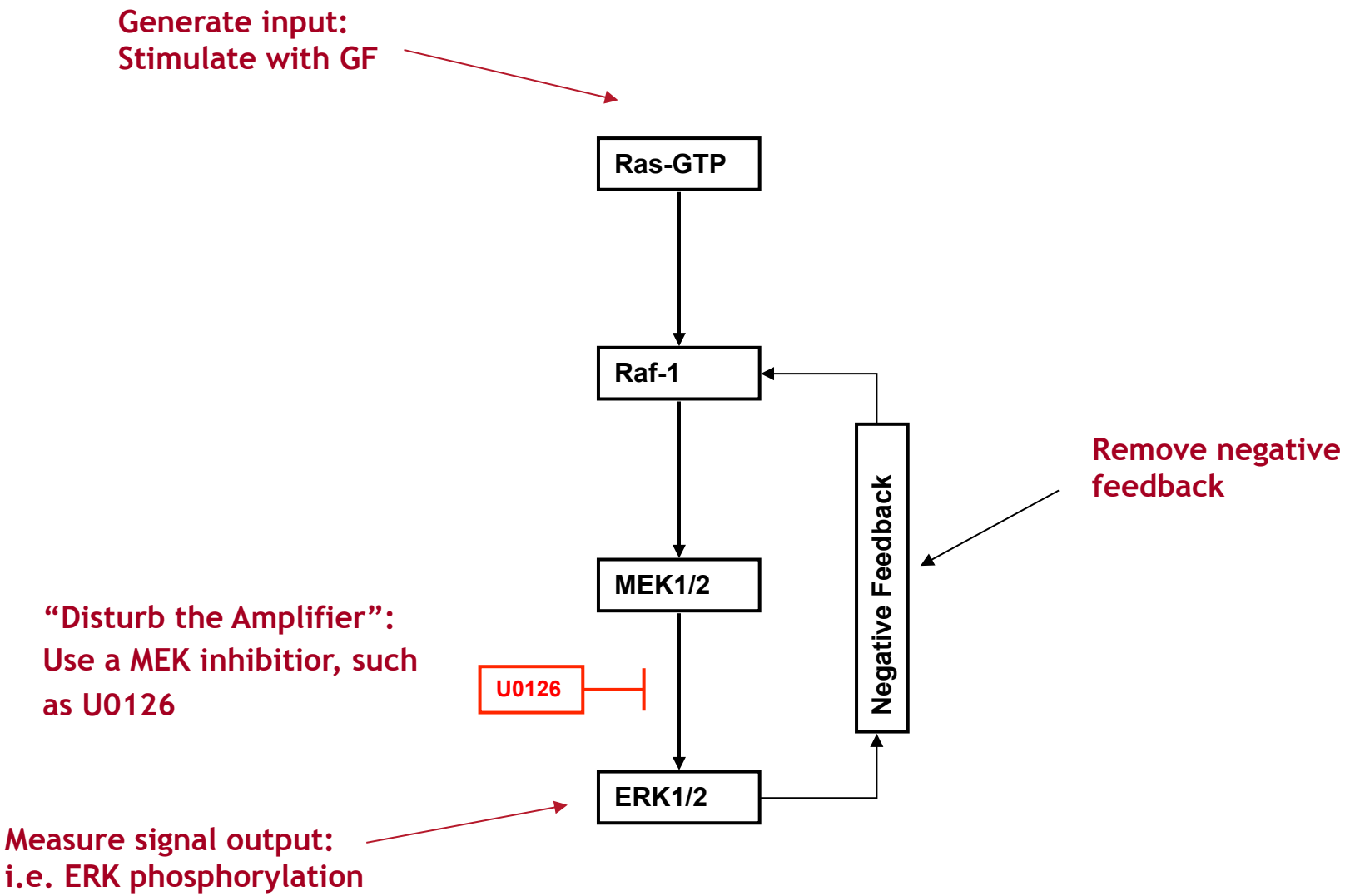
Sudden drop in Amplifier (A) gain



Sudden drop in Amplifier (A) gain

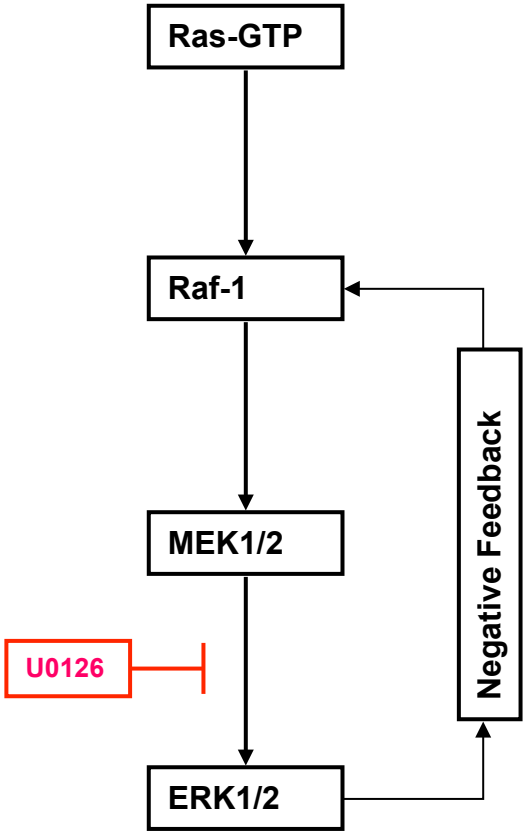


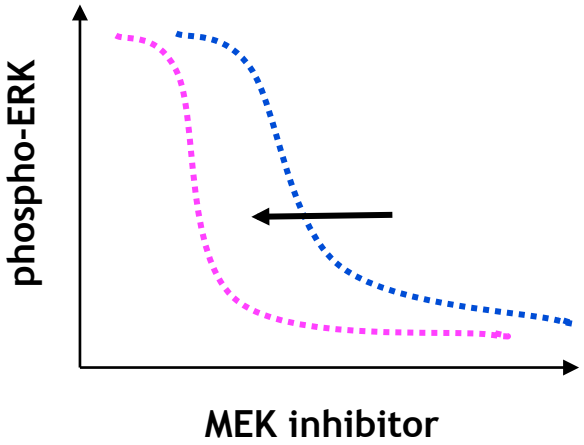
How to test if the ERK pathway is a NFA?



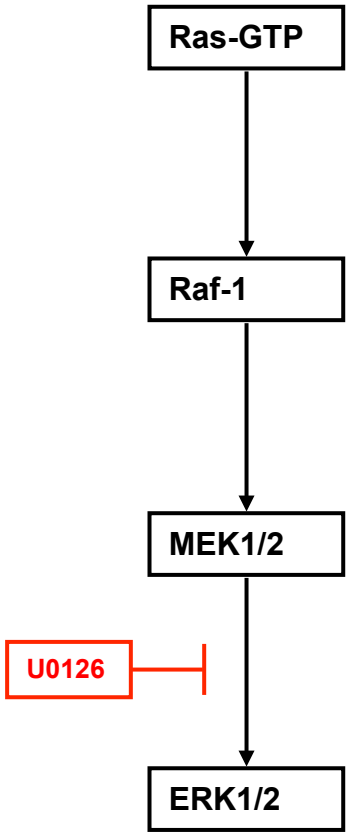
Hypothesis: Breaking the feedback should sensitise the ERK pathway to MEK-inhibitor

Feedback intact





Feedback removed



How to test if the ERK pathway is a NFA?

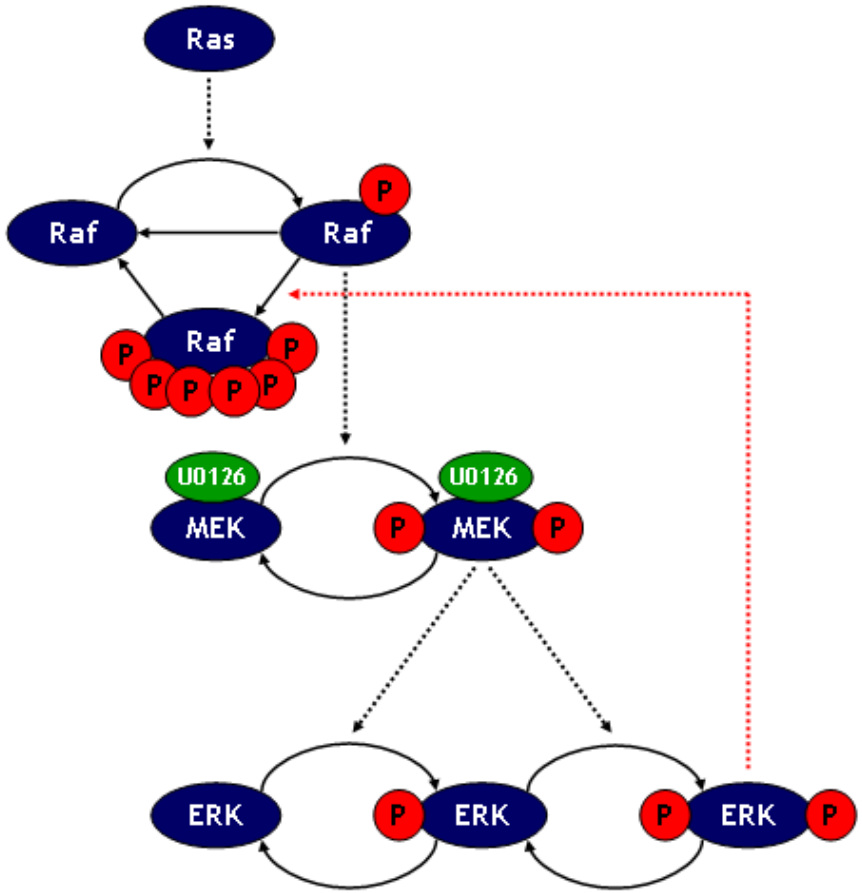
Strategy

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

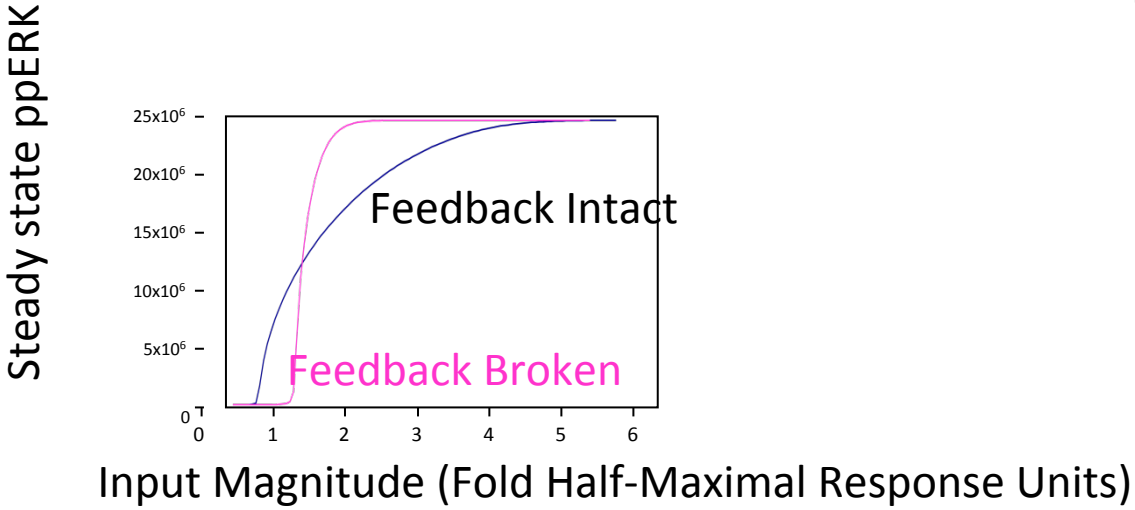
- Non-linear ordinary differential equations (ODE's).
- ODE's were solved using Math Lab and Gepasi.
- Models are based on the Schoeberl et al. (2002) model
- Mass Action Kinetics instead of Michaelis Menten
- Kinetic parameters are from literature, previous models and "guesstimates"



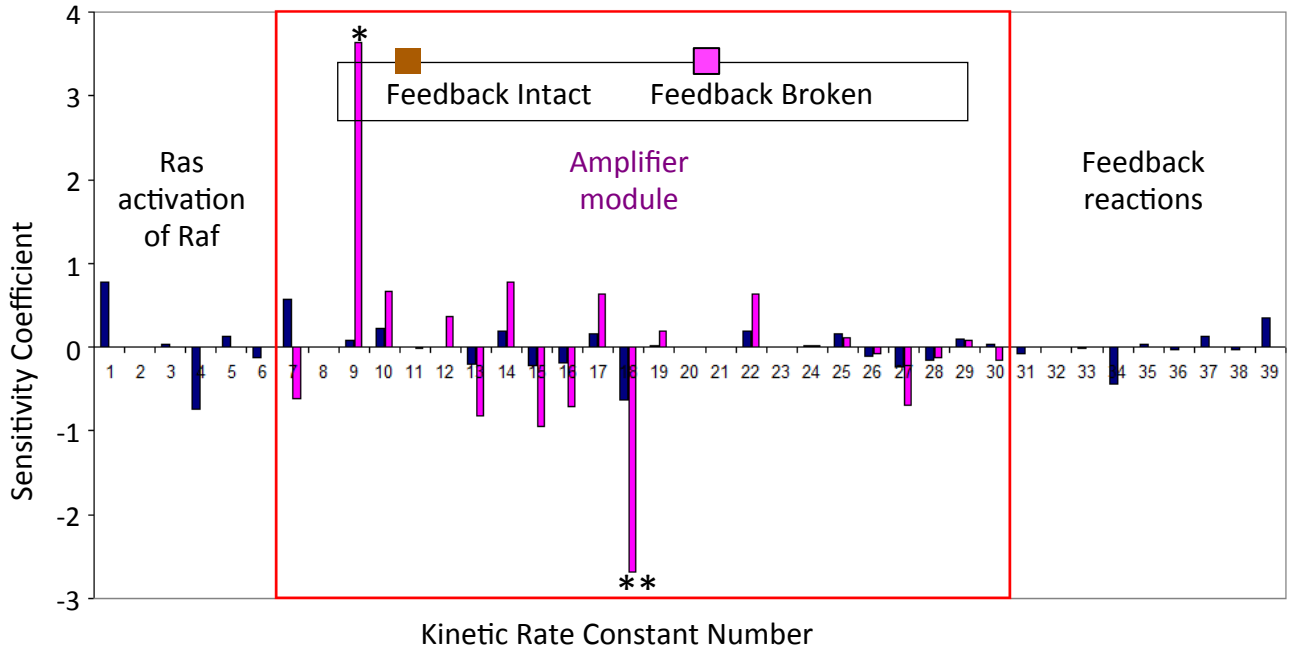
Schoeberl *et al*. (2002), Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors, Nature Biotechnology 20, 370-375

Figure 2

A)


Steady state ppERK
25x10⁶
20x10⁶
15x10⁶
10x10⁶
5x10⁶
0
0
1
2
3
4
5
6
Feedback Intact
Feedback Broken
Input Magnitude (Fold Half-Maximal Response Units)

B)


Sensitivity Coefficient
4
3
2
1
0
-1
-2
-3
*
**
Feedback Intact
Feedback Broken
Ras activation of Raf
Amplifier module
Feedback reactions
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
Kinetic Rate Constant Number

The experimental systems

Negative feedback loops intact

One feedback loop eliminated by constitutively active RasV12 mutant

Both feedback loops eliminated by BXB-ER (4-OHT regulatable Raf-1 mutant)

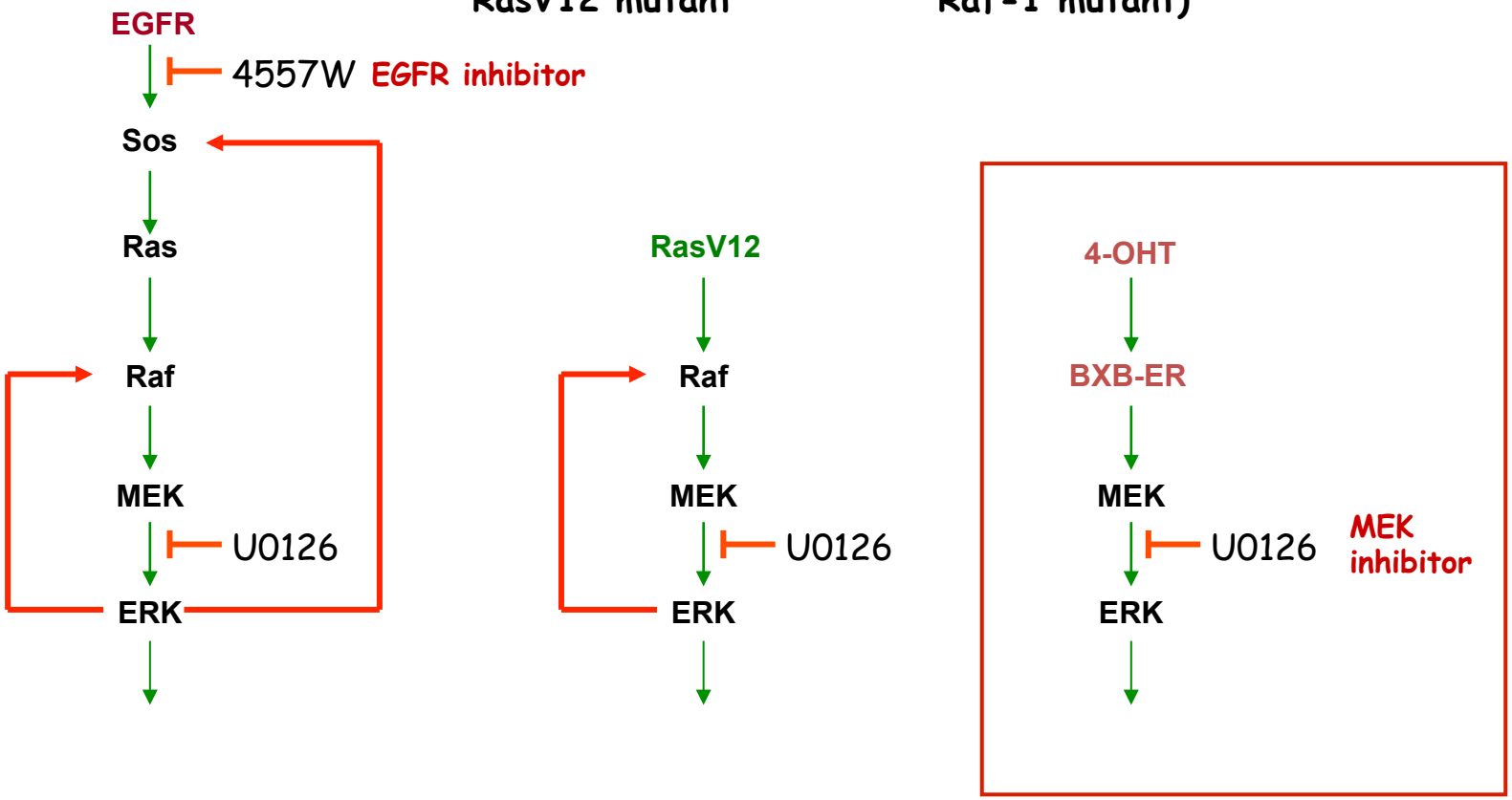
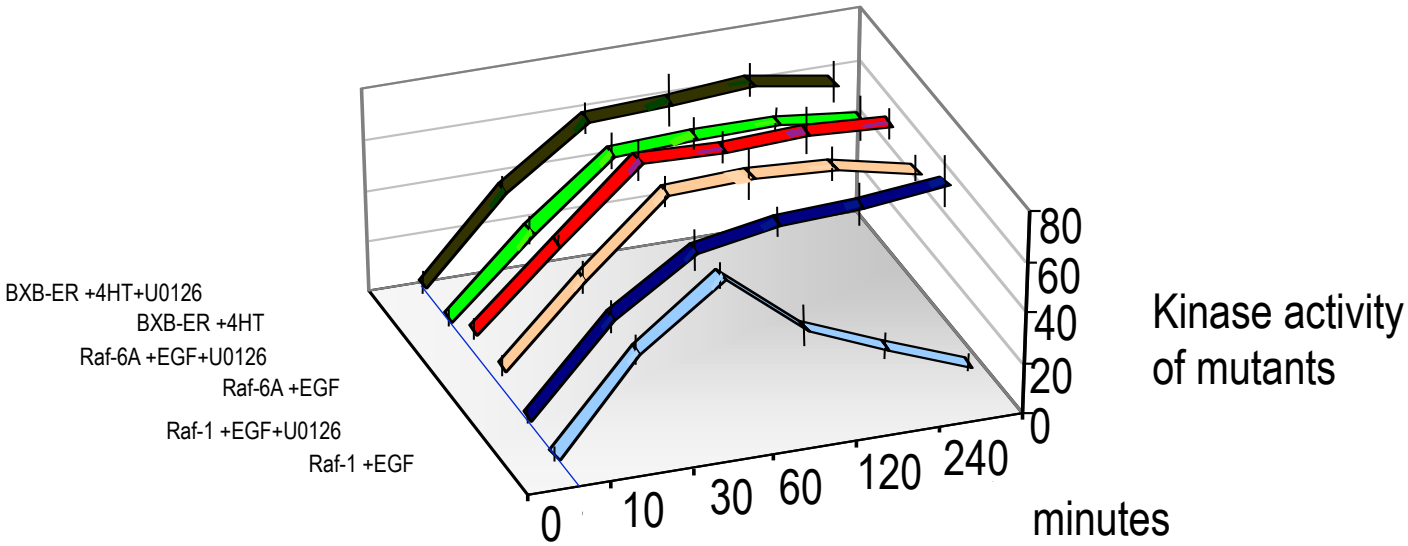
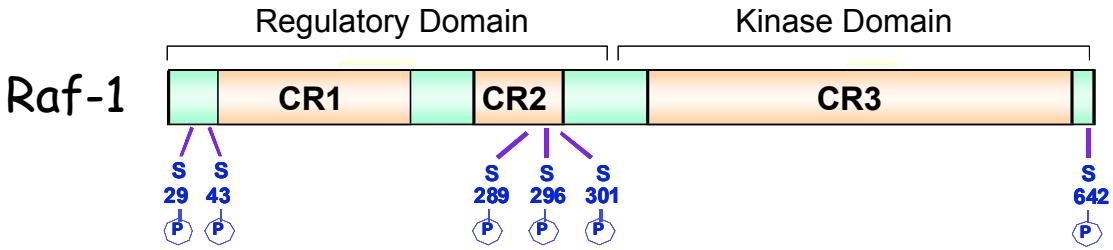


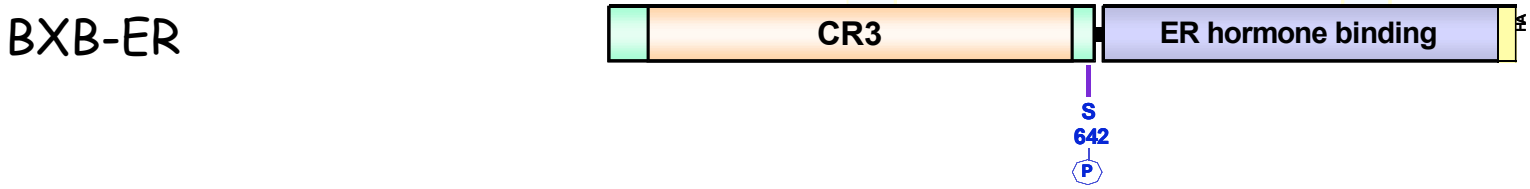
Figure 1

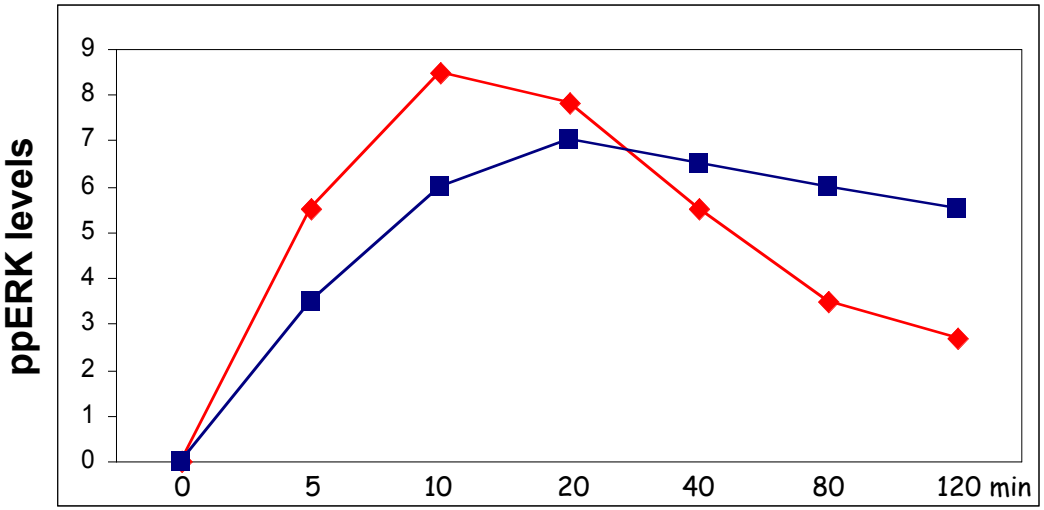

BXB-ER +4HT+U0126
BXB-ER +4HT
Raf-6A +EGF+U0126
Raf-6A +EGF
Raf-1 +EGF+U0126
Raf-1 +EGF
80
60
40
20
0
Kinase activity
of mutants
0
10
30
60
120
240
minutes

Breaking the ERK feedback with BXBER



ERK feedback phosphorylation sites



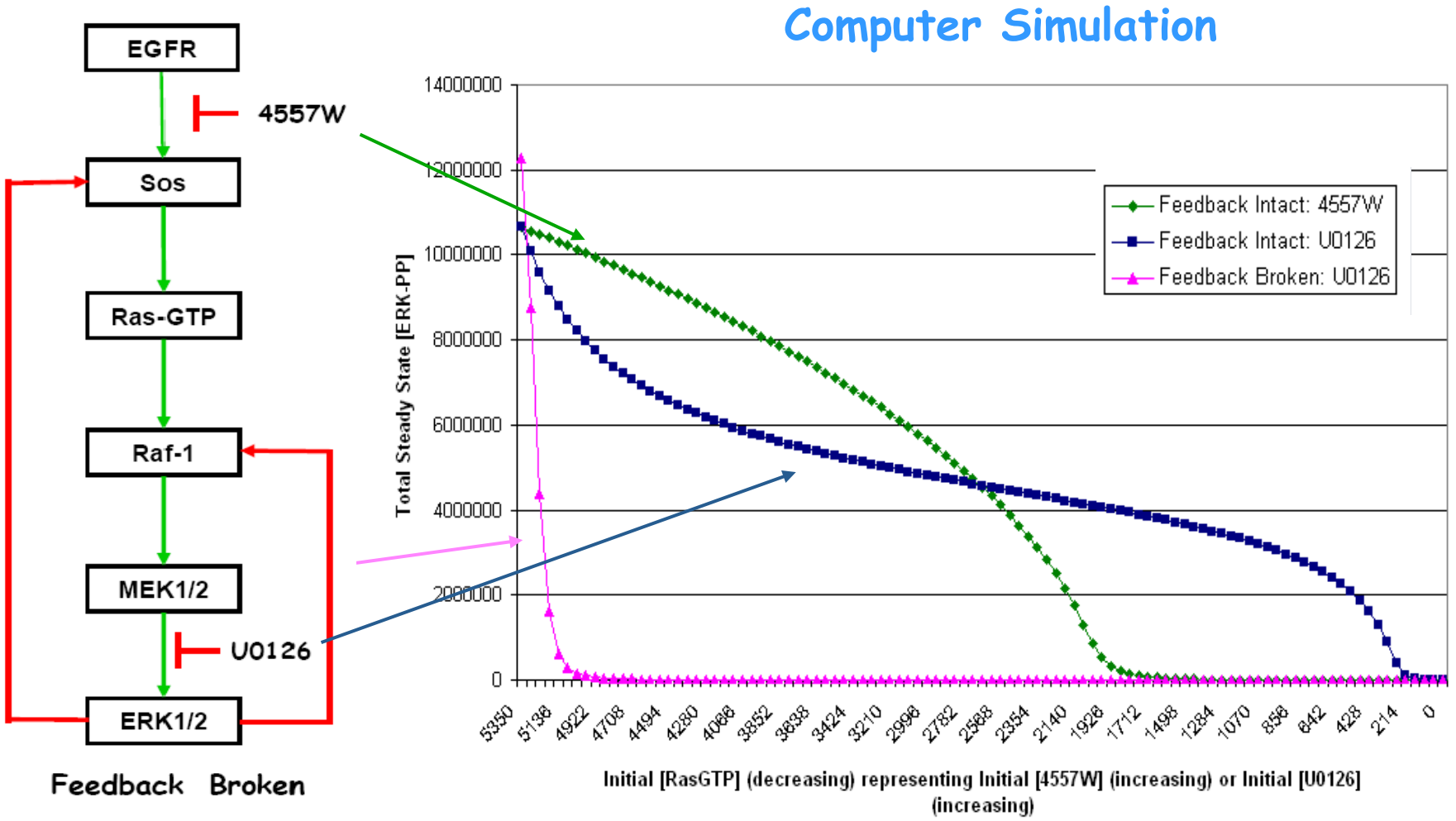


BXB-ER stimulated with 4-OHT

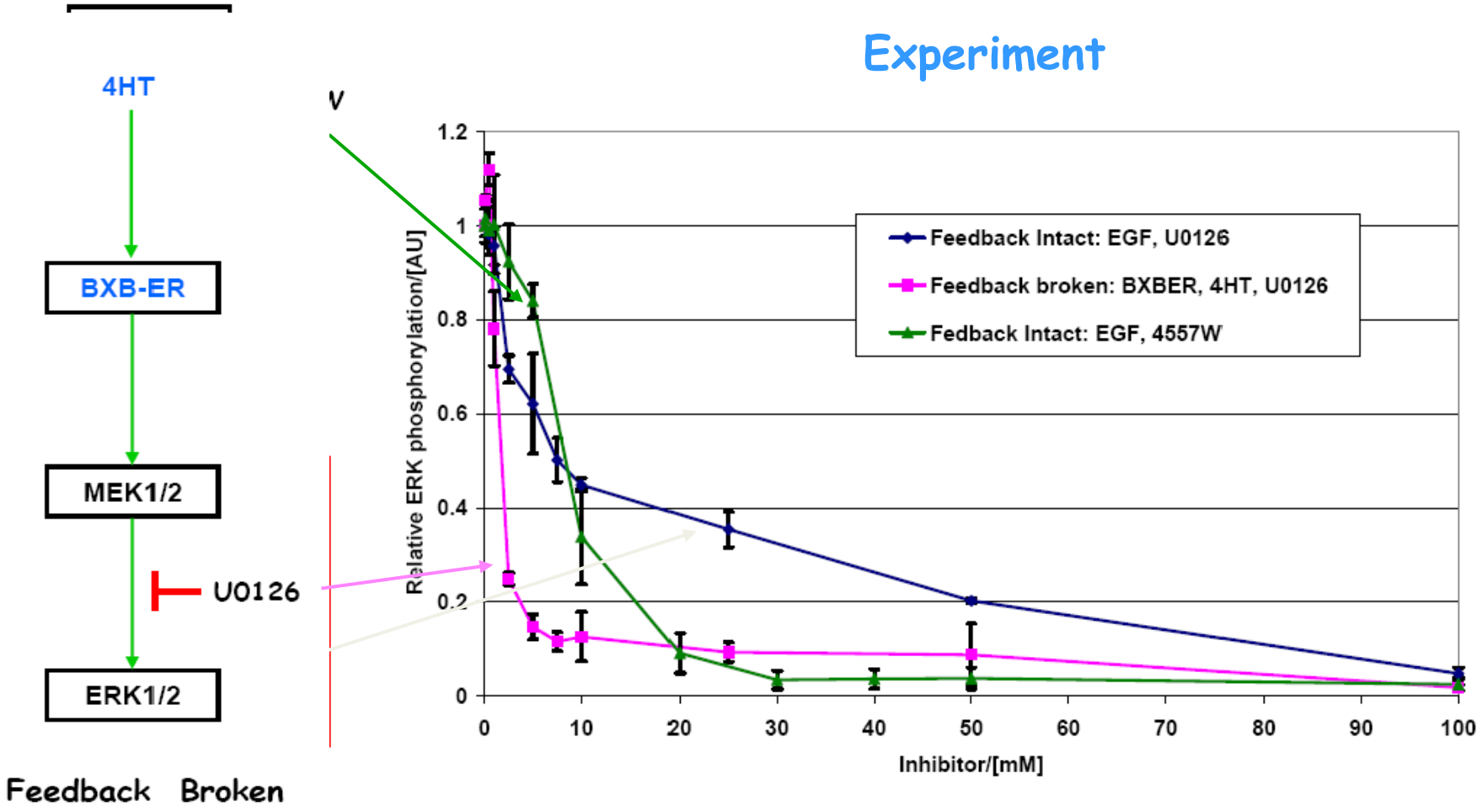
(4-Hydroxy Tamoxifen, a synthetic estrogen)

Raf-1 stimulated with EGF

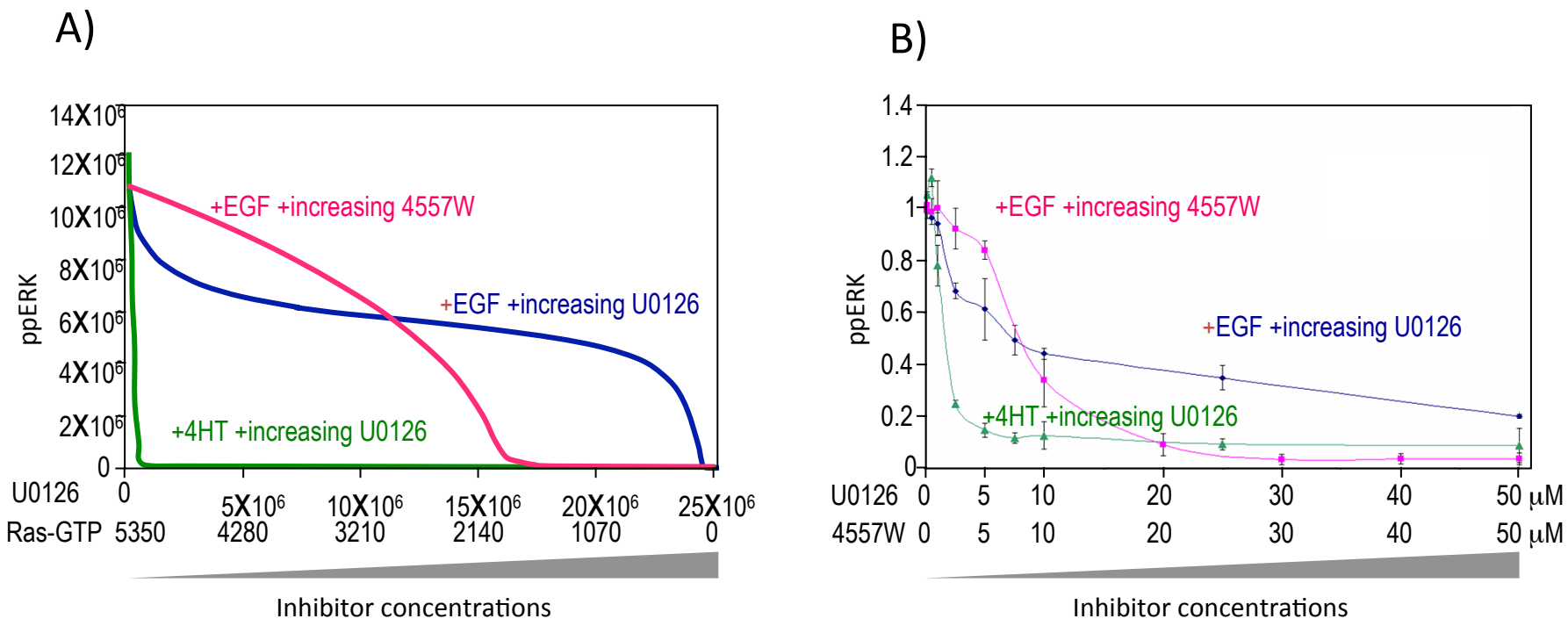
Ablation of feedback by BXBER decreases robustness to MEK-inhibitor U0126



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

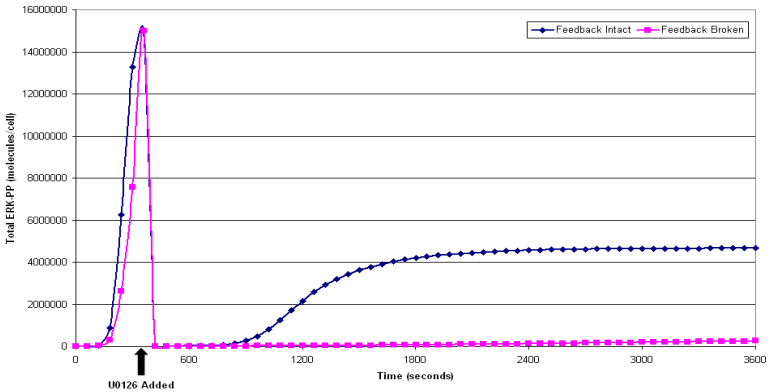


(A) Model prediction (B) Biochemical validation

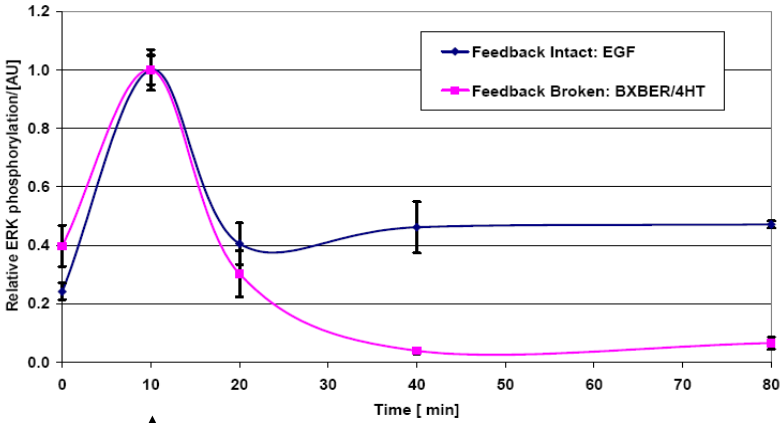


Signal recovery after MEK inhibition

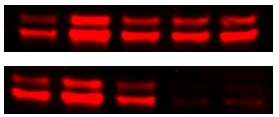
Simulation



Experiment



U0126 added

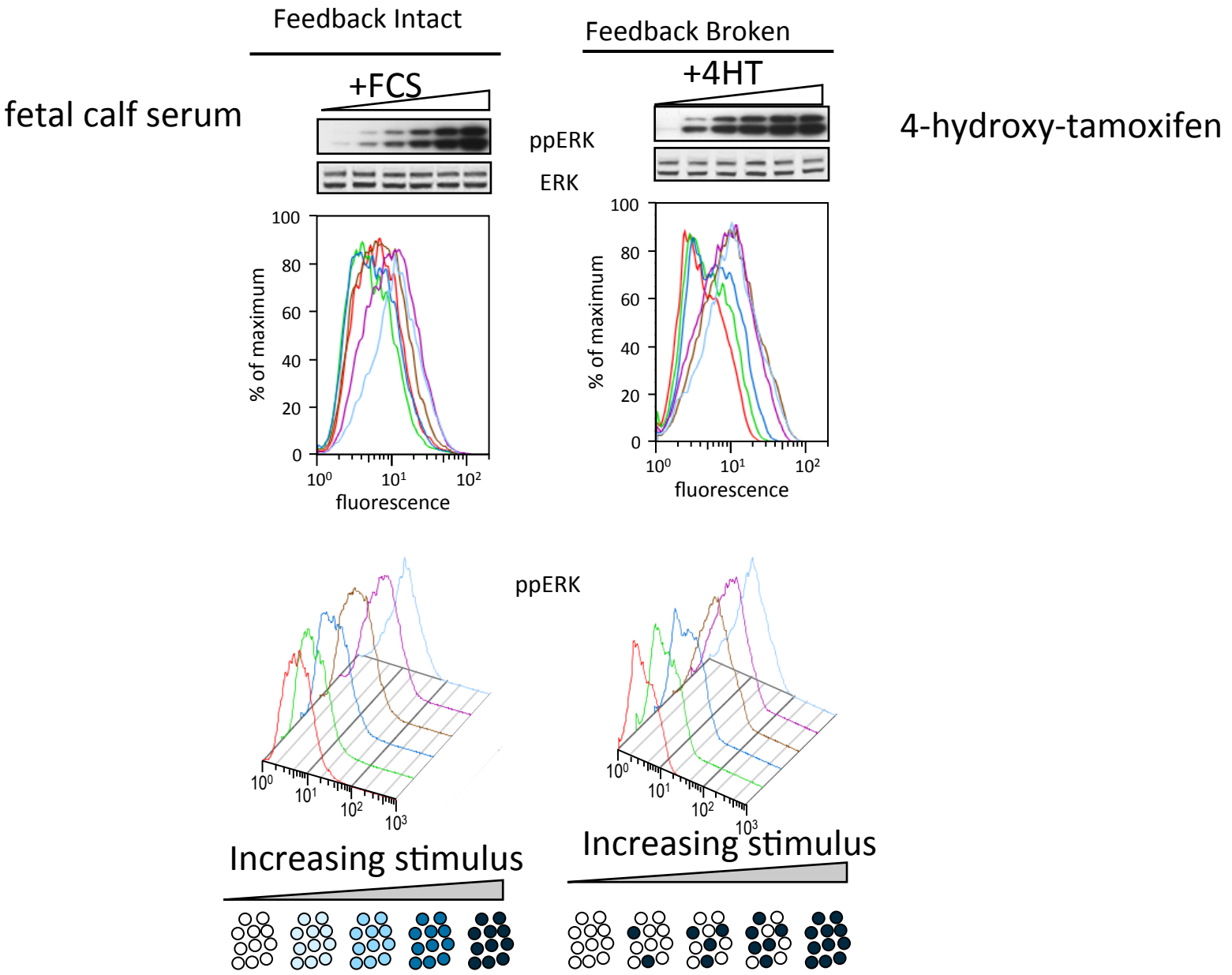


pERK1/2, +EGF

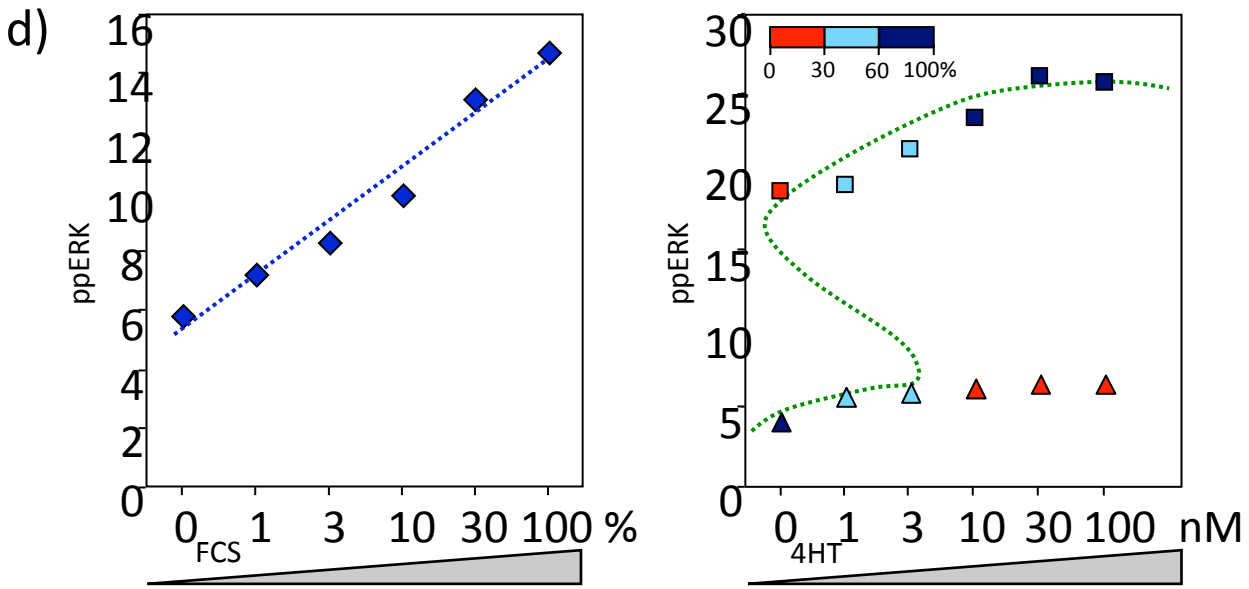
pERK1/2, + BXBER/4HT

0 10 20 40 80 min stimulation

Figure 2


Feedback Intact
Feedback Broken
+FCS
+4HT
fetal calf serum
4-hydroxy-tamoxifen
ppERK
ERK
% of maximum
fluorescence
ppERK
Increasing stimulus
Increasing stimulus

Graded or switch-like?



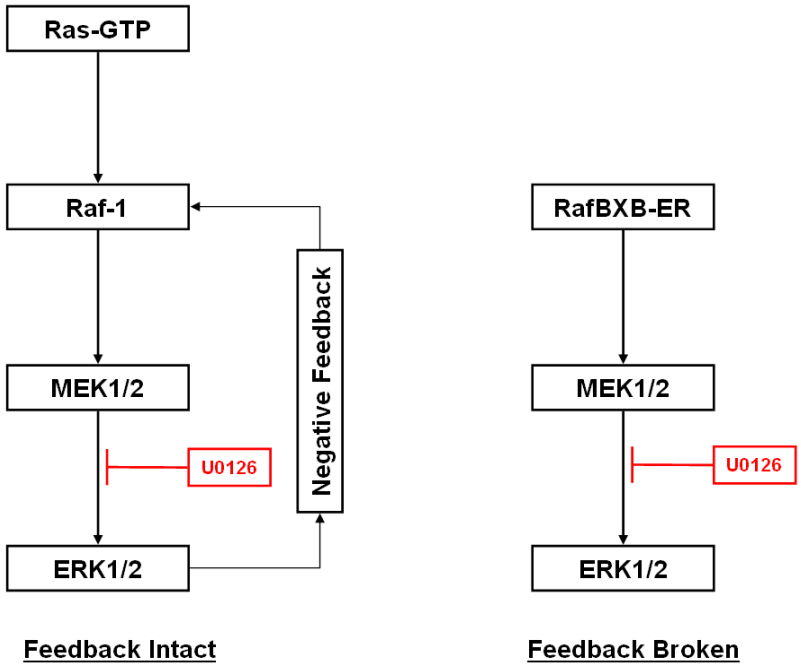
•The presence of the negative feedback dictates whether ERK activation follows a graded or switch-like pattern.

•Demonstrated by the differences in ERK activation responses when the negative feedback is broken

•The biological NFA provides a mechanism to generate analog or digital responses.

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)



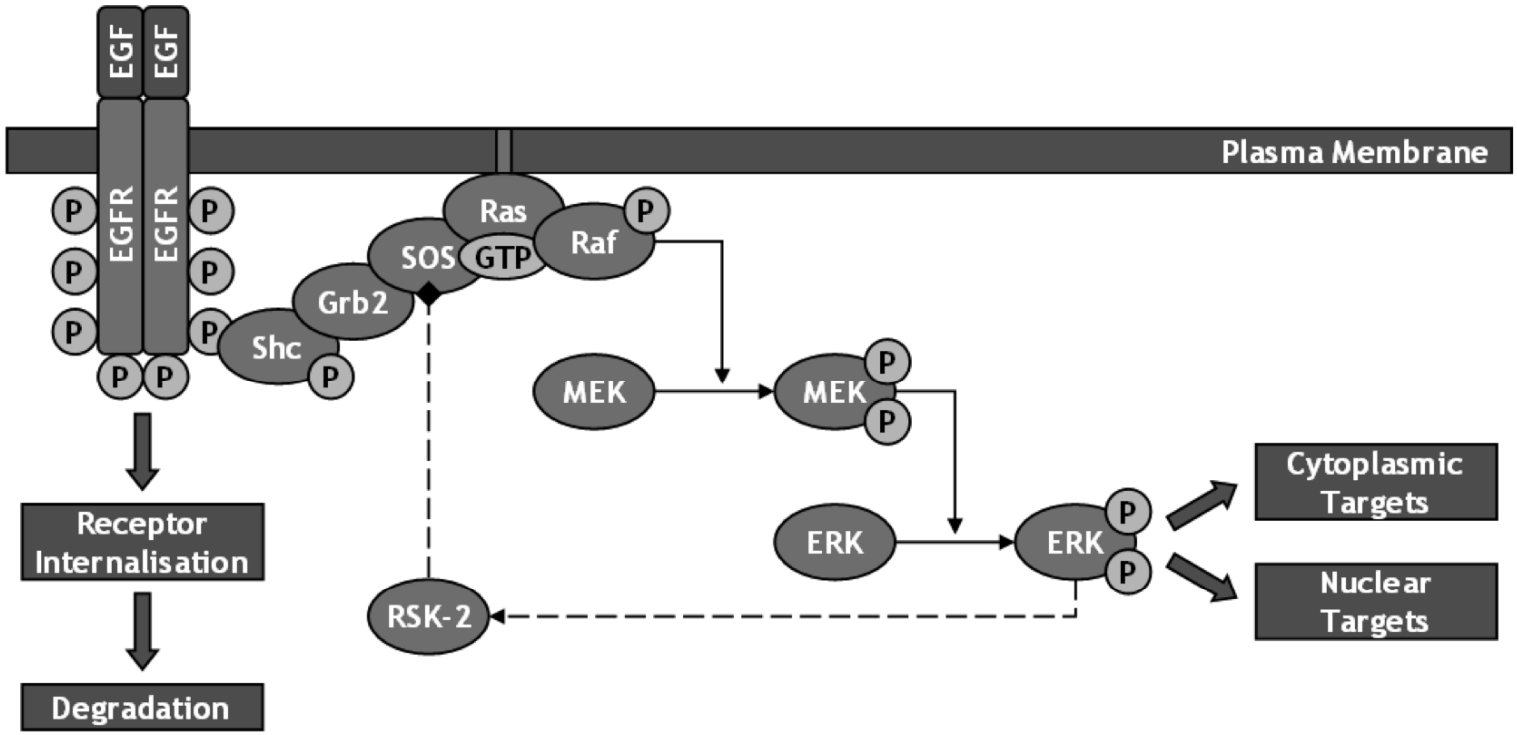
The Mammalian MAPK/ERK Pathway Exhibits Properties of a Negative Feedback Amplifier

- Three-tiered kinase module, signal amplifier.
- Negative feedback loops - system like negative feedback amplifier
- Smoothens the output to changes in input - system robust to change.
- No feedback loops: cells sensitive to inhibition of MEK
- Feedback intact: cells are resistant to inhibition there. D

- **Drug development: inhibitors targetting components outside NFA are more effective at inhibiting the pathway.**

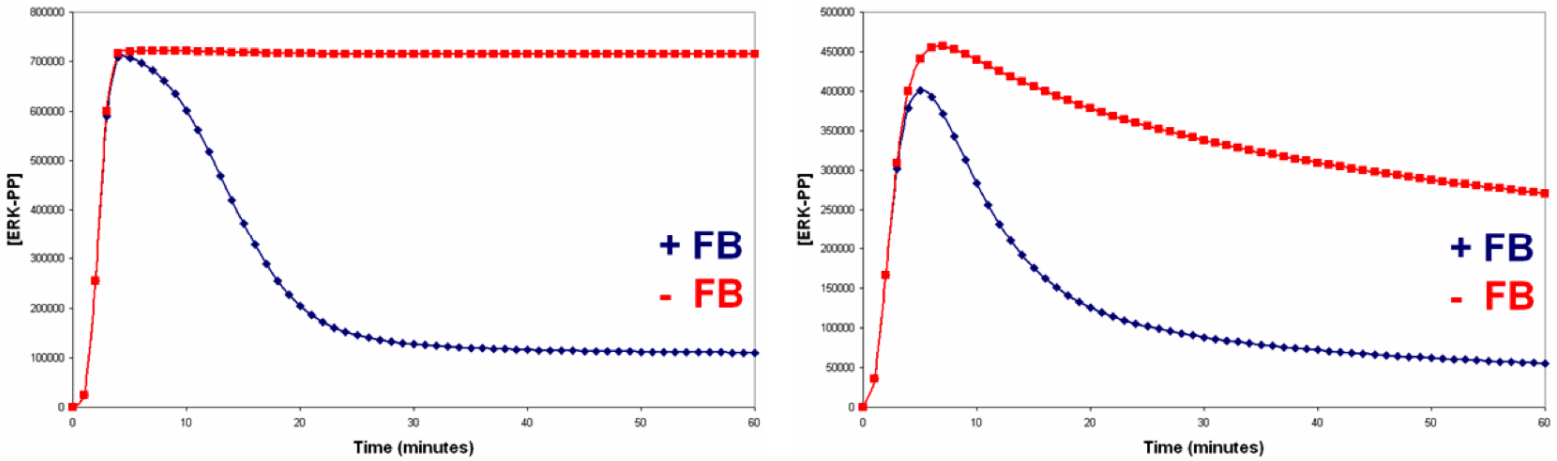
Sturm, Orton, Vyshemirsky, Grindlay, Birtwistle, Gilbert, Calder, Pitt, Kholodenko and Kolch., Science Signalling Dec 21;3

Computational modelling reveals feedback redundancy within the EGFR/ERK signalling pathway



Orton, Sturm, Gormand, Kolch & Gilbert 2008. IET Systems Biology, 2:4, pp. 173 – 183

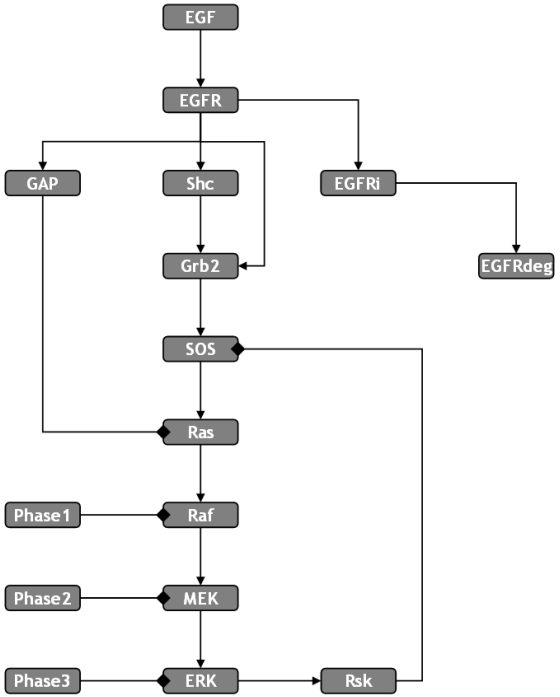
Computational modelling reveals feedback redundancy within the EGFR/ERK signalling pathway

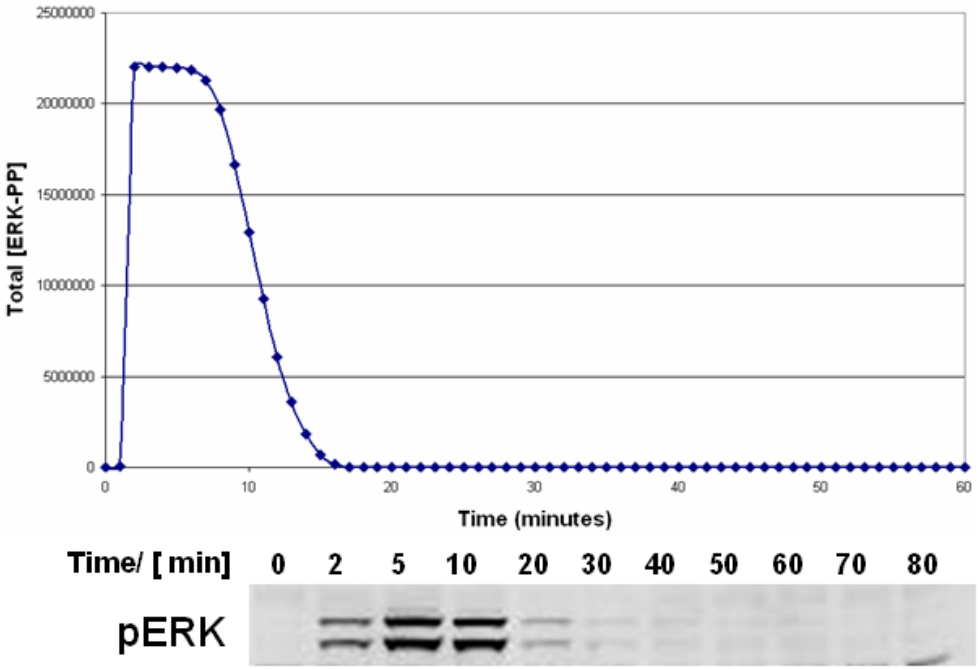


- **Simulations of the Brightman and Brown models with and without the SOS negative feedback loop present**
- *in both cases knocking out the negative feedback loop has a dramatic effect with the ERK-PP signal switching from a transient to a sustained response, suggesting that the feedback loop is essential for the transient response and efficient signal termination.*

Orton, Sturm, Gormand, Kolch & Gilbert 2008. IET Systems Biology, 2:4, pp. 173 – 183

Computational modelling reveals feedback redundancy within the EGFR/ERK signalling pathway

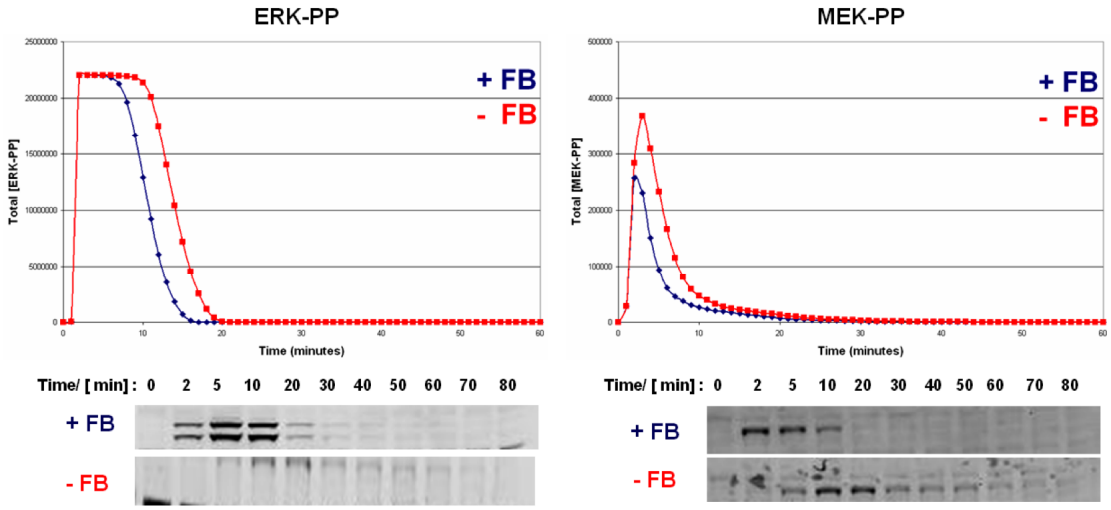




New model - Corrected version of Schoeberl model - utilises a receptor-complex strategy, receptor internalisation and degradation, and Shc-dependent and independent pathways leading to the activation of Ras.

Orton, Sturm, Gormand, Kolch & Gilbert 2008. IET Systems Biology, 2:4, pp. 173 – 183

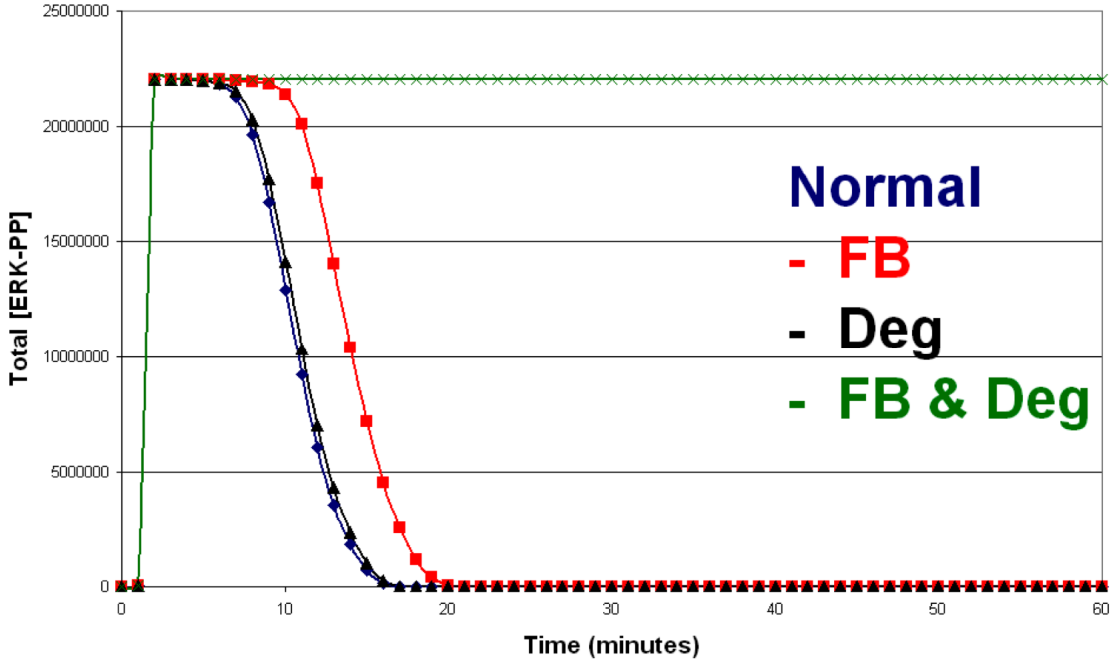
Computational modelling reveals feedback redundancy within the EGFR/ERK signalling pathway



- *Knocking out the feedback loop gives a slightly prolonged but still transient MEK signal – validates model's prediction that the feedback loop is not needed for the transient response*
- *The apparent delay in MEK activation is caused by increased vulnerability to phosphatase activity due to U0126 interference*

Orton, Sturm, Gormand, Kolch & Gilbert 2008. IET Systems Biology, 2:4, pp. 173 – 183

Computational modelling reveals feedback redundancy within the EGFR/ERK signalling pathway



- ***Individually*** *knocking out the feedback loop or receptor degradation has little effect as the ERK response still remains very much transient.*
- *Knocking out* ***both*** *the feedback loop and receptor degradation causes the response to switch from transient to sustained.*

Orton, Sturm, Gormand, Kolch & Gilbert 2008. IET Systems Biology, 2:4, pp. 173 – 183

Model checking in Systems Biology
Robin Donaldson

- Biologists will often talk in qualitative or semi-quantitative language (trends).
  - "this protein peaks after 5 minutes, then falls to half concentration"
  - Often quite certain about time.

- Systems biology; Part of model design process, validate the model conforms to the **observed** data.

- Synthetic biology; Make sure the model and constructed bio system conform to the **desired** behaviour.

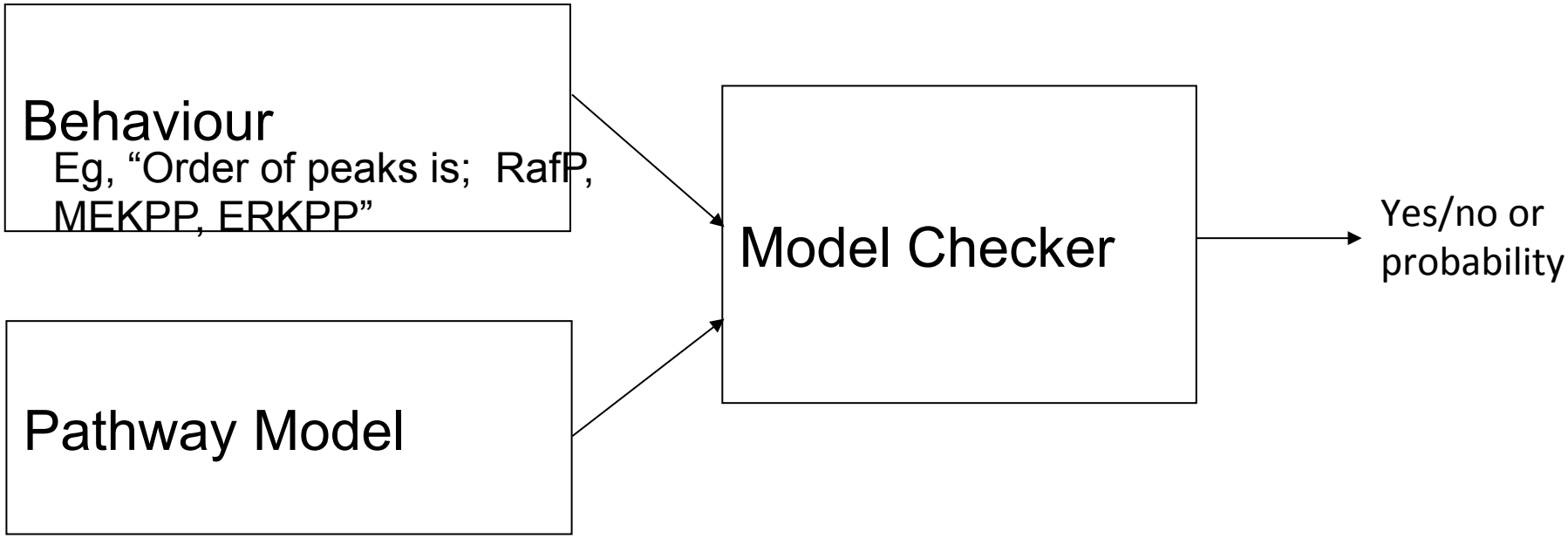
What can we do with model checking in sys/syn bio?

- **Model validation**:
  - Show that your model of the pathway matches the lab data
  - Show that the (constructed) biosystem conforms to the specification
  - May not be obvious behaviours, so not easy to see by eye!
  - Might have a high probability of doing what you want, but doesn't always do it!

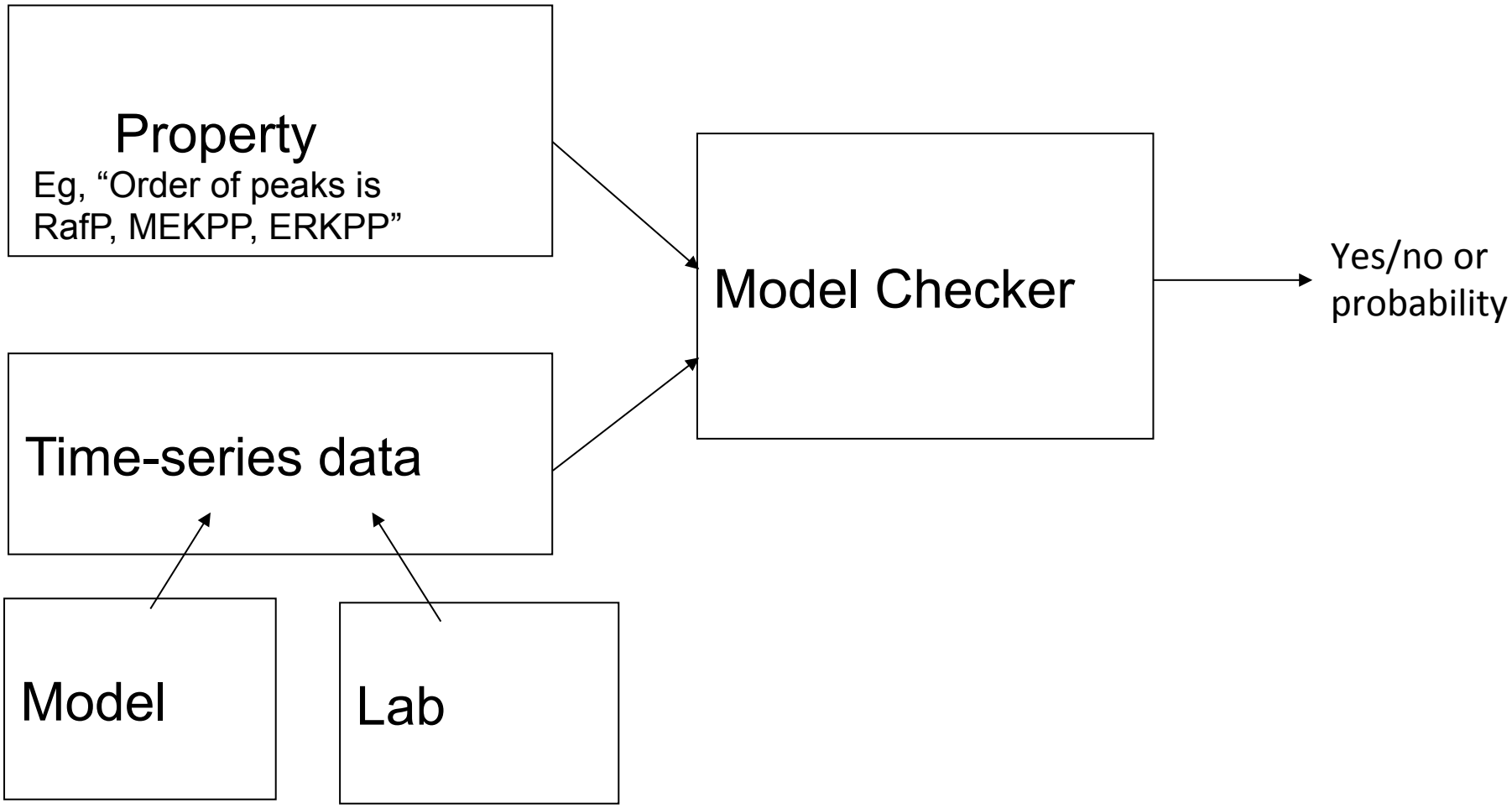
- **Model building**:
  - If the model doesn't do what we want, we can change the model (automatically?) until it does!
  - Change the parameters of a model (reaction rates/initial concentrations) until the pathway behaves as you want

- **Model finding**:
  - Many models in a database, can use PLTL as a query language like SQL.
  - "Give me all the models in the database which oscillate"

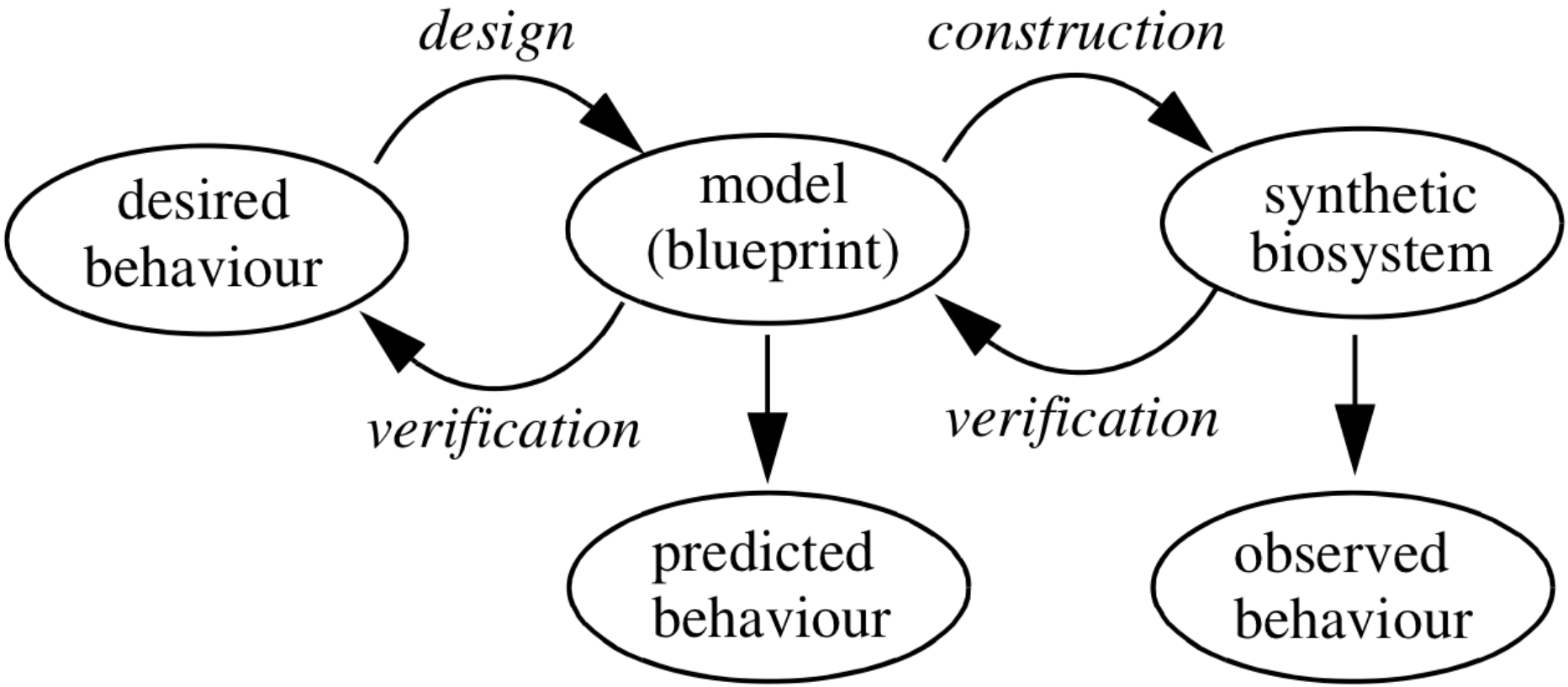
Model Checking Biochemical Pathways



Time-series based Model Checking of Biochemical Pathways



Synthetic Biology



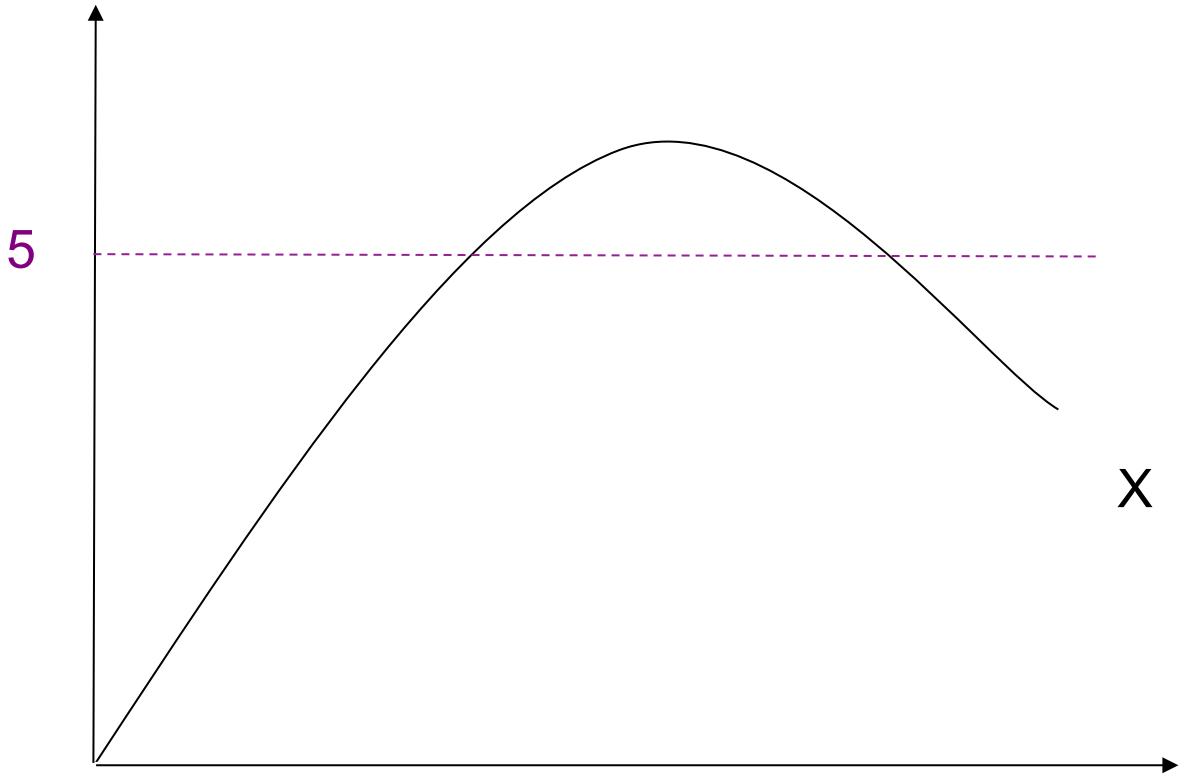
MC2 model checker

- Simulative, off-line
- Trace set can be:
  - Set of stochastic runs
  - A single continuous run
  - A parameter scan
  - Lab data!
- Simulation output from;
  - ODE, SDE, CTMC, Gillespie, hybrid approaches, multi-cellular simulation, open models
- Experimental data from the wet lab

MC2 with ODE Output

$P_{=?}[\ F(\ X > 5\ )\ ]$

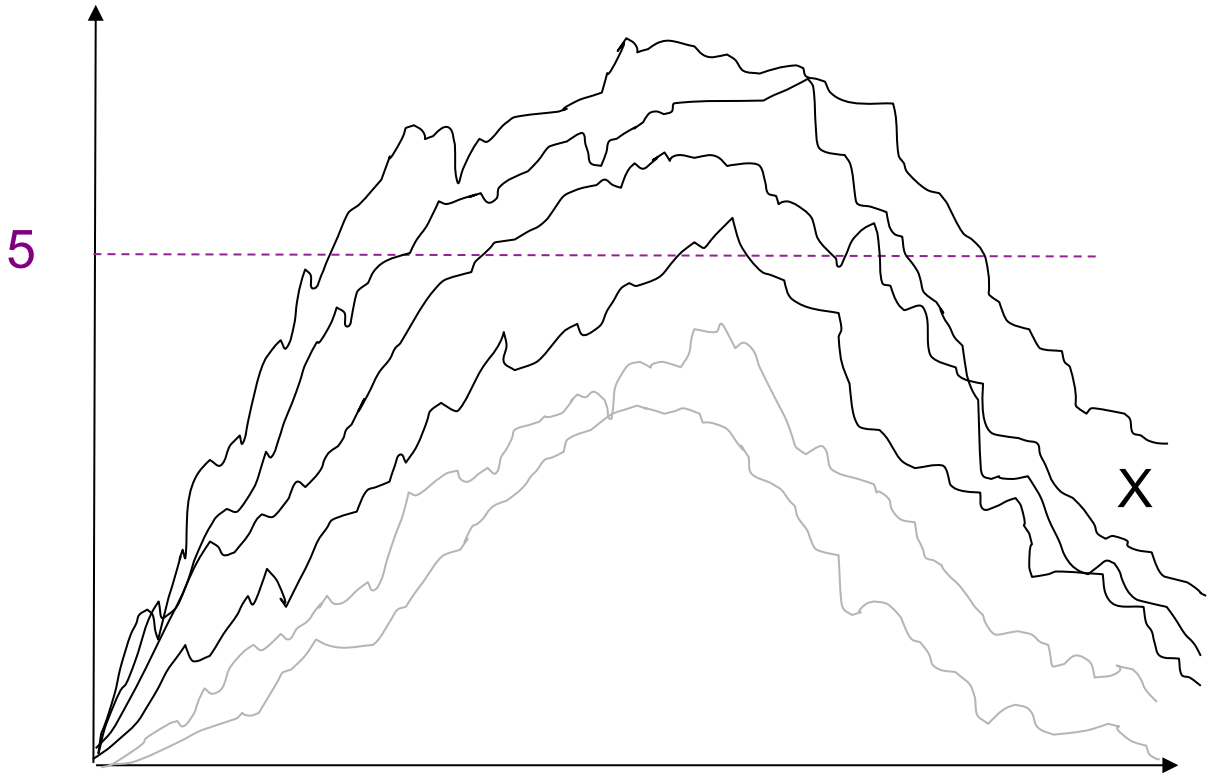
=> P = 1



MC2 with Gillespie Output

$$P_{=?}[\ F(\ X > 5\ )\ ]$$

=> P = 4/6



PLTL language

- Behaviours to be checked against a model is expressed in temporal logic

- We chose:
  Probabilistic logic called Probabilistic Linear-time Temporal Logic (PLTL)

- Main PLTL operators:

  G (P) – P always happens

  F (P) – P happens at some time

  X (P) – P happens in the next time point

  (P1) U (P2) – P1 happens until P2 happens

  P1 { P2 } – P1 happens from the first time P2 happens

Range of expressivity in PLTL

- **Qualitative**:
  Protein rises then falls
  P=? [(d(Protein) > 0) U (G(d(Protein) < 0))]

- **Semi-qualitative**:
  Protein rises then falls to less than 50% of peak concentration
  P=? [(d(Protein) > 0) U (G(d(Protein) < 0) ∧ F ([Protein] < 0.5 ∗ max[Protein]))]

- **Semi-quantitative**:
  Protein rises then falls to less than 50% of peak concentration by 60 minutes
  P=? [(d(Protein) > 0) U (G(d(Protein) < 0) ∧ F (time = 60 ∧ Protein < 0.5 ∗ max(Protein)))]

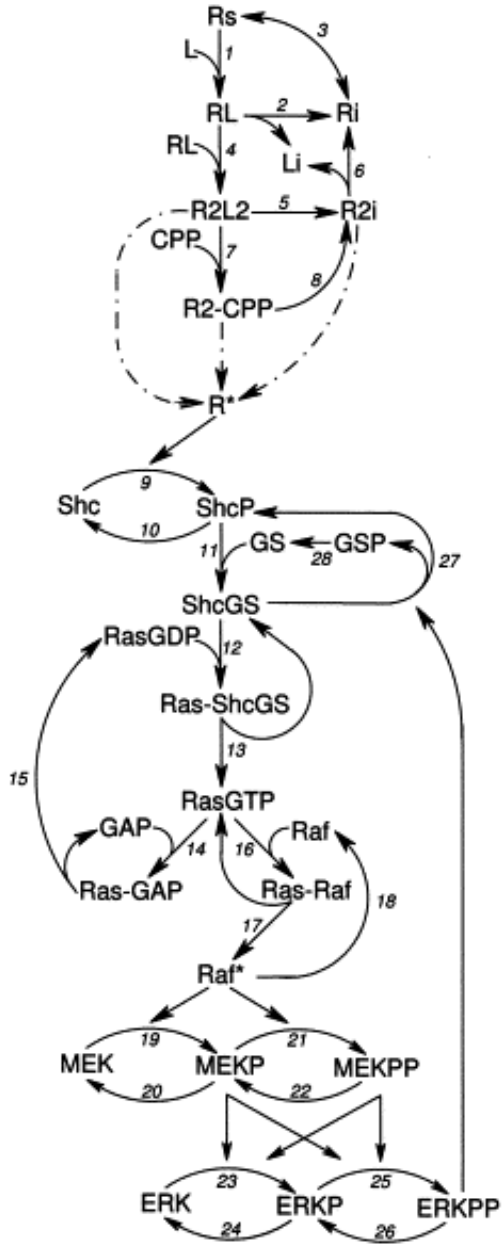
- **Quantitative**:
  Protein rises then falls to less than 100µMol by 60 minutes
  P=? [(d(Protein) > 0) U (G(d(Protein) < 0) ∧ F (time = 60 ∧ Protein < 100))]

Model development: Parameter estimation

Continuous Brightman & Fell model:

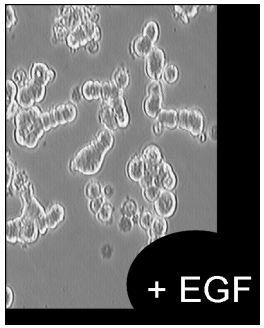
The EGF signal transduction pathway produces transient Ras, MEK and ERK activation whereas NGF stimulation produces sustained activation.

Parameter V28 has the the highest probability of generating the desired behaviour, but requires 40-fold increase in value



Brightman & Fell, FEBS Lett 2000. "Differential feedback regulation of the MAPK cascade underlies the quantitative differences in EGF and NGF signalling in PC12 cells"

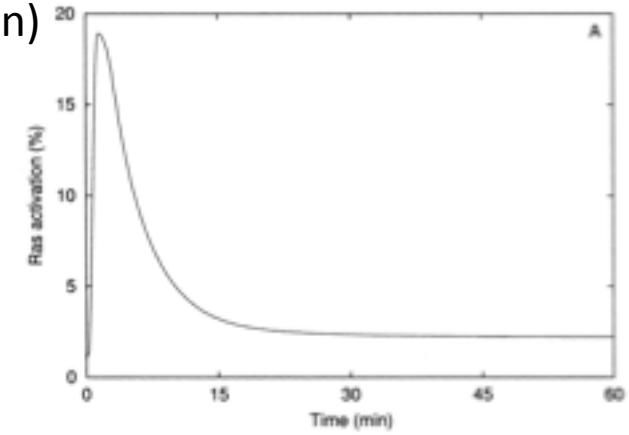
Parameter estimation
Response with EGF vs. NGF signal

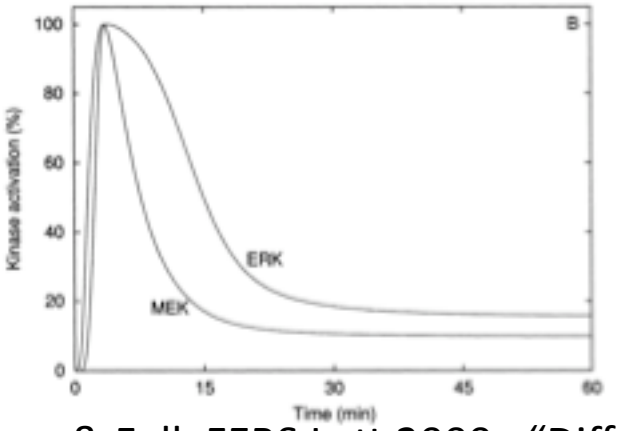


Proliferation
(cell division)
PC12 cells

EGF

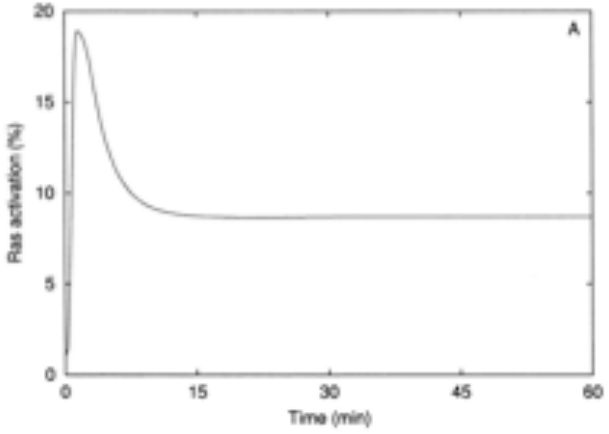
Transient activation of Ras, MEK and ERK

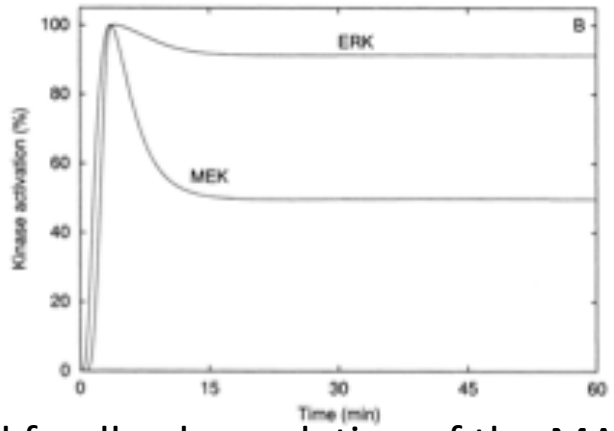


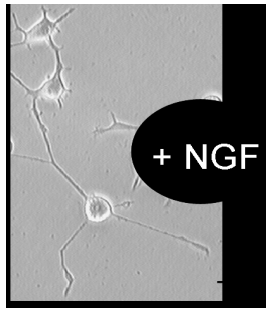


NGF

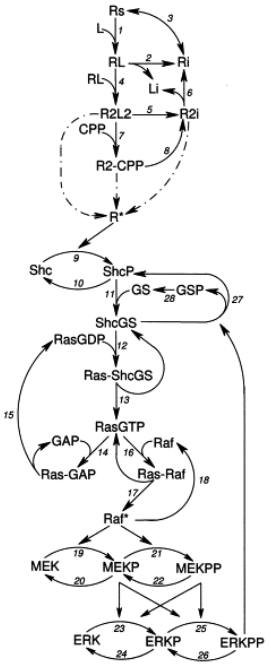
Sustained activation of Ras, MEK and ERK







Differentiation
(neurite
outgrowth)



Brightman & Fell, FEBS Lett 2000. "Differential feedback regulation of the MAPK cascade underlies the quantitative differences in EGF and NGF signalling in PC12 cells"

Desired Behaviour in PLTLc

The desired (sustained) NGF behaviour of the pathway was written in the original model paper.
Can be written in PLTLc as:

Sustained Ras: Active Ras peaks within 2 minutes to a maximum of 20% of total Ras and is stable between 5% and 10%

$P_{=?}$ [d(active Ras) > 0 U (time ≤ 2 ⋀ active Ras ≥ 0.15∗total Ras
⋀ active Ras ≤ 0.2∗total Ras ⋀ (d(active Ras) < 0)
U (G(active Ras ≥ 0.05∗total Ras ⋀ active Ras ≤ 0.10∗total Ras)))]

Sustained MEK: Active MEK peaks within 2 to 5 minutes and is stable between 40% and 50% of peak value

$P_{=?}$ [d(MEKPP) > 0 U (time ≥ 2 ⋀ time ≤ 5 ⋀ d(MEKPP) < 0
U (G(MEKPP ≥ 0.40∗max(MEKPP) ⋀ MEKPP ≤ 0.50∗max(MEKPP))))]

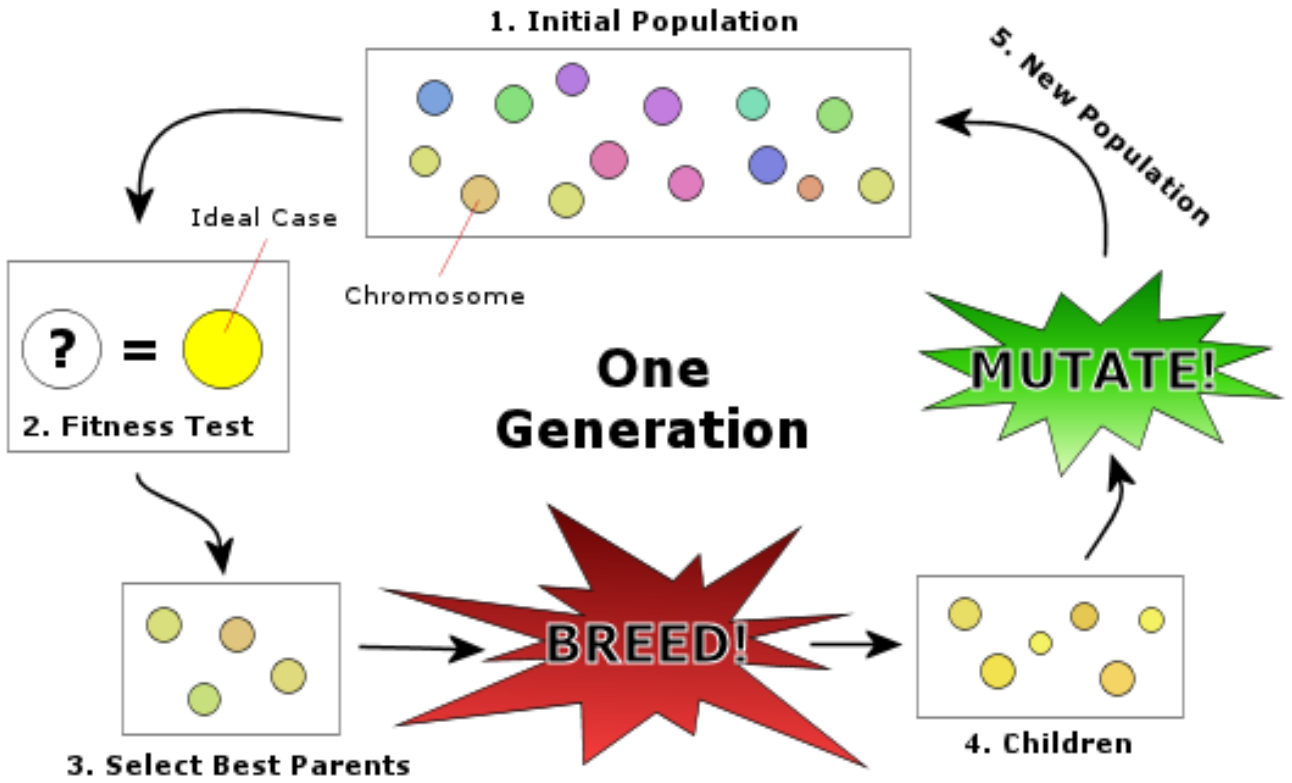
Sustained ERK: Active ERK peaks within 2 to 5 minutes and is stable between 85% and 100% of peak value

$P_{=?}$ [(d(ERKPP) > 0) U (time ≥ 2 ⋀ time ≤ 5 ⋀ d(ERKPP) < 0
U (G(ERKPP ≥ 0.85 ∗ max(ERKPP))))]

Robin Donaldson and David Gilbert (2008). A Model Checking Approach to the Parameter Estimation of Biochemical Pathways In proceedings CMSB 2008 (Computational Methods in Systems Biology). To Appear.

Model construction using a genetic algorithm

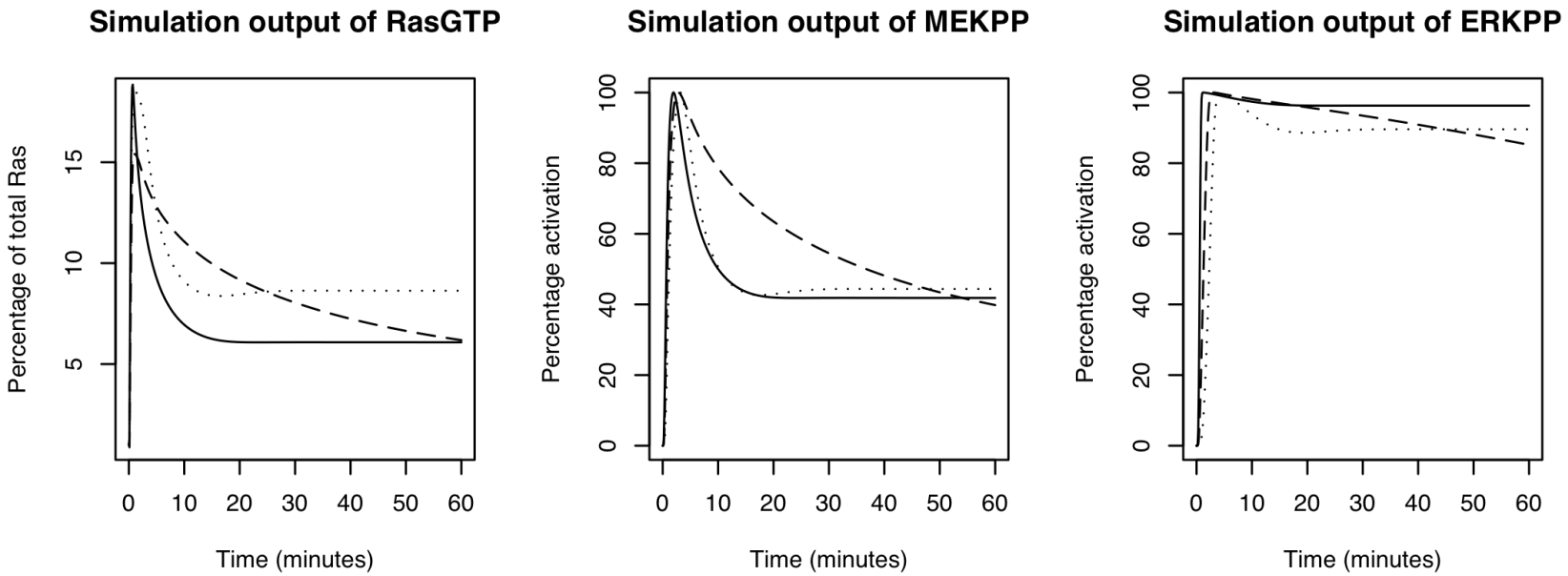
Sustained ERK: Active ERK peaks within 2 to 5 minutes and is stable between 85% and 100% of peak value



2000 models, 100 generations: 200,000 simulations/checks

Parameter fitting results

- Built a fitness function for sustained Ras, MEK and ERK
- Ran the genetic algorithm with 100 generations and obtained results:

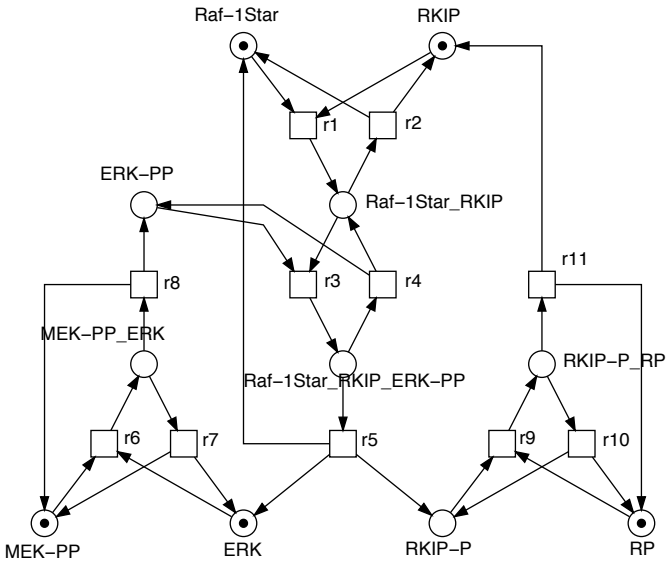


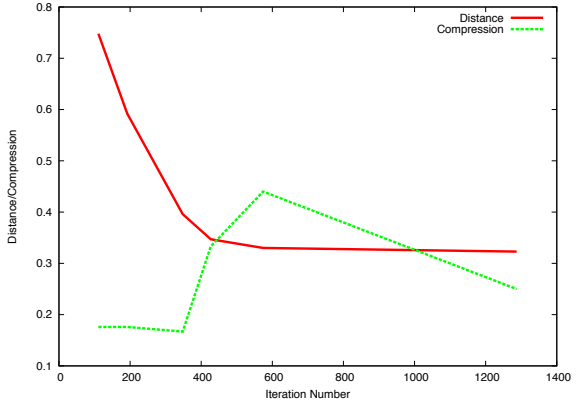
- Original model of the NGF signalling pathway varying V28 (dotted)
- Best model returned when varying the critical parameters (solid)
- Critical parameters without V28 (dashed).

The best model returned when varying the critical parameters only required a ***16-fold*** increase in V28 (compared with 40-fold in original paper)

Even possible to get similar behaviour **without** varying V28

Target Driven Biochemical Network Reconstruction Based on Petri Nets and Simulated Annealing

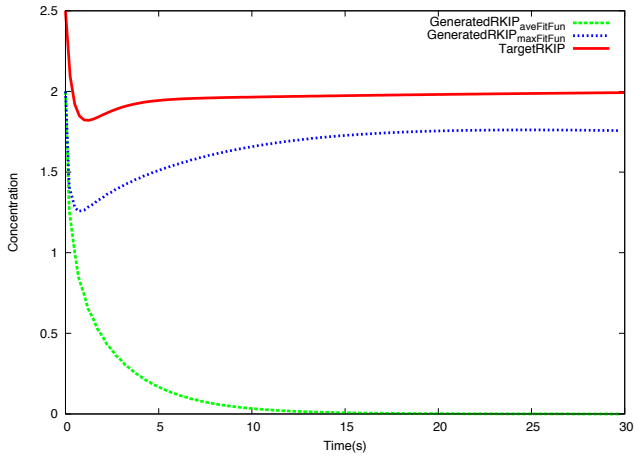




$$FitFun = f\left(\sqrt{\sum_{i=1}^{m}([P_{GT}]_i - [P_{TT}]_i)^2}\right)$$

Table 1: Basic Enzymatic Reactions

Name	Chemical Equation
ER_1	$RKIPP + Raf1 \rightleftharpoons RKIPP\|Raf1 \rightarrow RKIPP' + Raf1$
ER_2	$ERKPP + Raf1 \rightleftharpoons ERKPP\|Raf1 \rightarrow ERKPP' + Raf1$
ER_3	$RKIP + Raf1 \rightleftharpoons RKIP\|Raf1 \rightarrow RKIPP + Raf1$
ER_4	$ERK + Raf1 \rightleftharpoons ERK\|Raf1 \rightarrow ERKP' + Raf1$
ER_5	$RKIPP + MEKPP \rightleftharpoons RKIPP\|MEKPP \rightarrow RKIPP' + MEKPP$
ER_6	$ERKPP + MEKPP \rightleftharpoons ERKPP\|MEKPP \rightarrow ERKPP' + MEKPP$
ER_7	$RKIP + MEKPP \rightleftharpoons RKIP\|MEKPP \rightarrow RKIPP + MEKPP$
ER_8	$ERK + MEKPP \rightleftharpoons ERK\|MEKPP \rightarrow ERKP' + MEKPP$
ER_9	$RKIPP + RP \rightleftharpoons RKIPP\|RP \rightarrow RKIPP' + RP$
ER_{10}	$ERKPP + RP \rightleftharpoons ERKPP\|RP \rightarrow ERKPP' + RP$
ER_{11}	$RKIP + RP \rightleftharpoons RKIP\|RP \rightarrow RKIPP + RP$
ER_{12}	$ERK + RP \rightleftharpoons ERK\|RP \rightarrow ERKP' + RP$

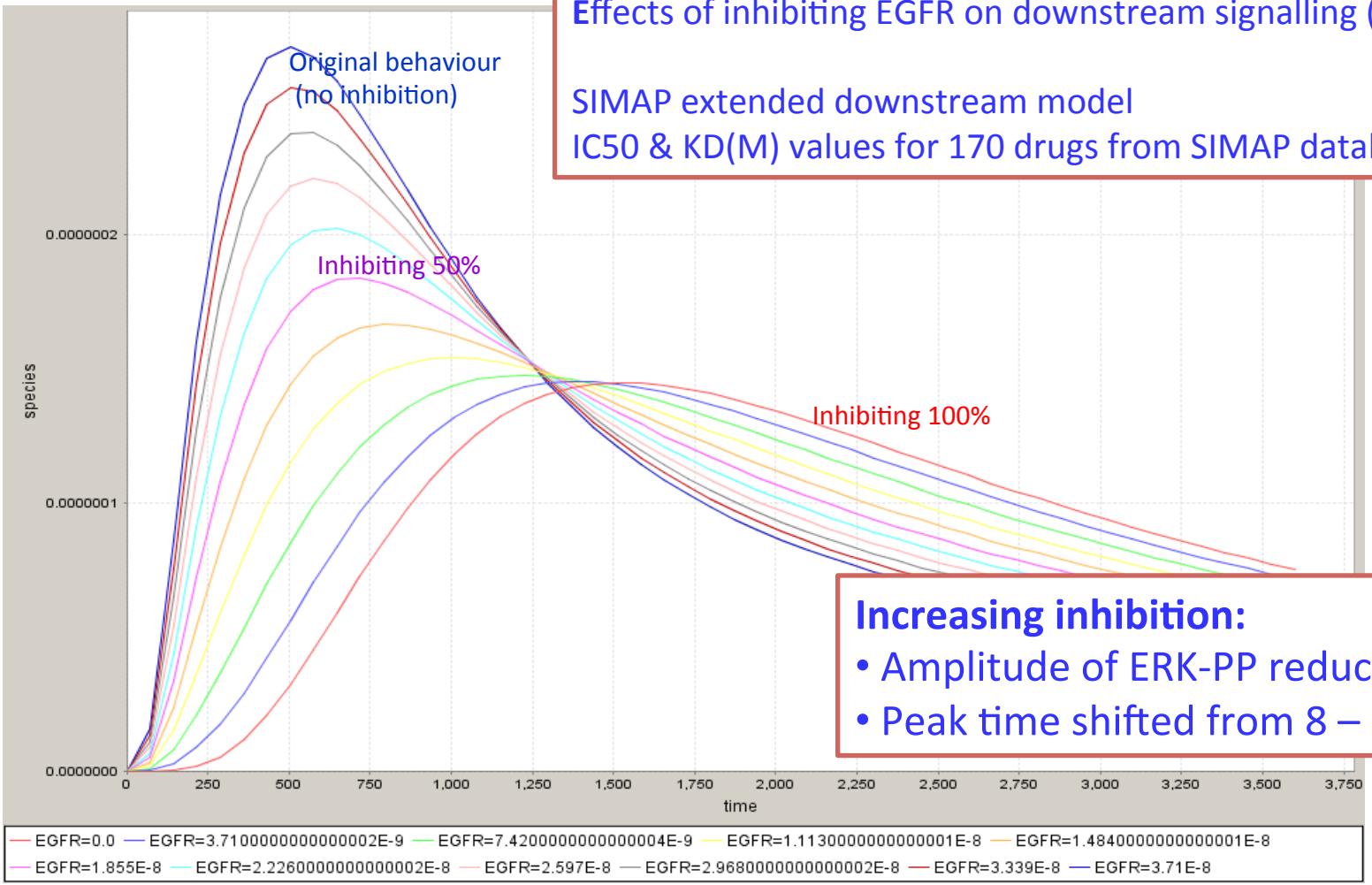


Wu, Gao & Gilbert. CMSB 2010

Modelling the effect of drug inhibition
- towards individualised patient models

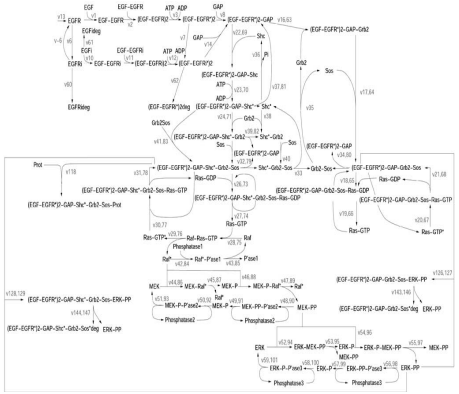
Effects of inhibiting EGFR on downstream signalling (ERK-PP).

SIMAP extended downstream model
IC50 & KD(M) values for 170 drugs from SIMAP database



Increasing inhibition:

- Amplitude of ERK-PP reduced
- Peak time shifted from 8 – 25 mins

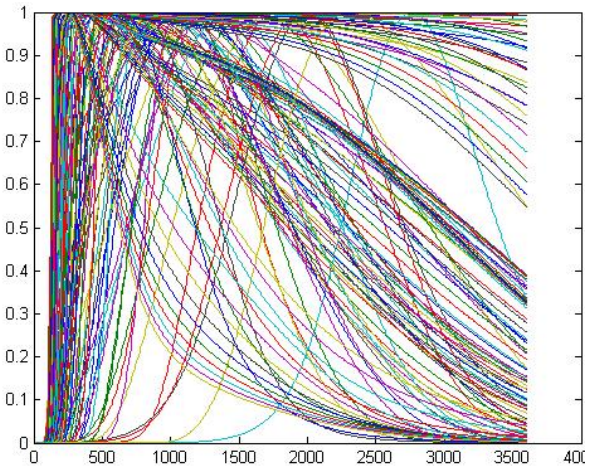


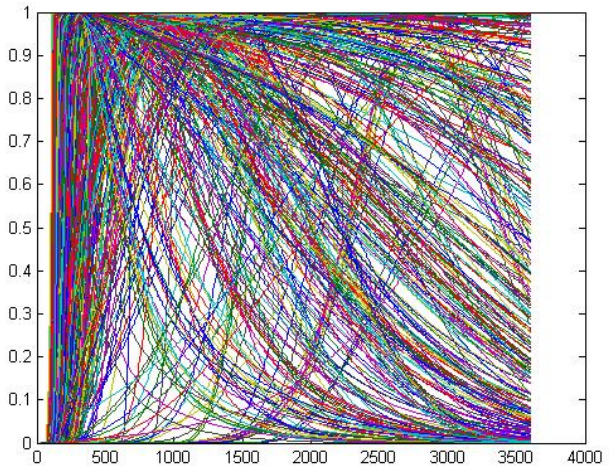
Modelling & Analysing knock-downs

Pam Gao, MAPK Pathway

- What multiple knockdowns are **interesting**?
- Problem – many possible combinations
- Very time-consuming & expensive to carry out all possible assays
- Model – Alter for each k/d, simulate, analyse

Single k/d
11 steps



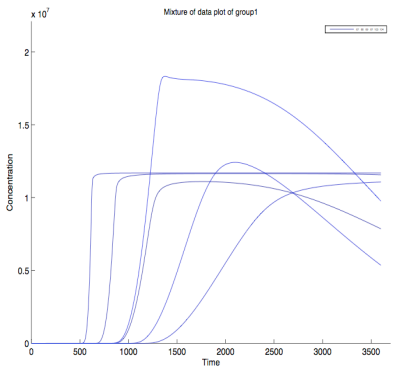


Double k/d,
3 steps

Concentration of ERK-PP (activated ERK) over time : 60mins

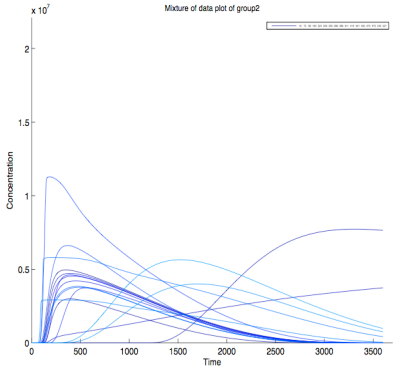
Clustering of multiple knock-downs

Delayed



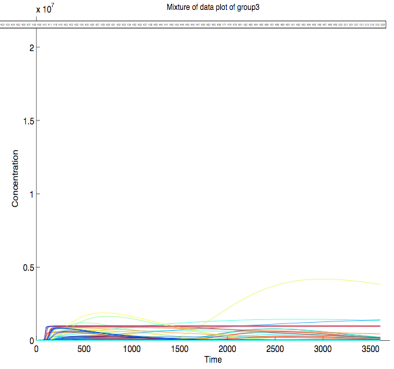
Cluster 1

Amplitude reduced, delayed



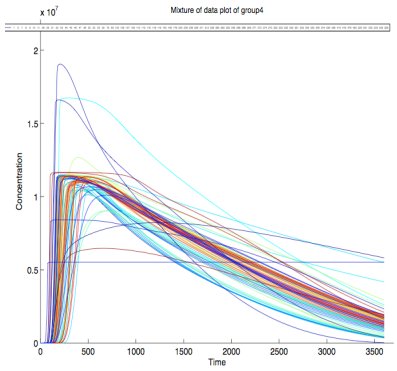
Cluster 2

Low activation levels



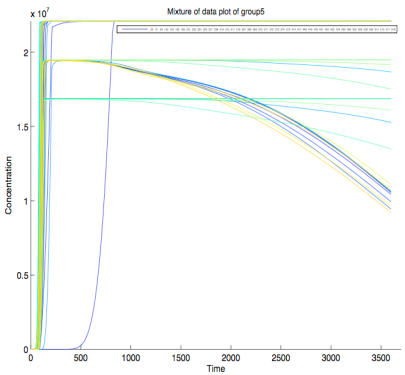
Cluster 3

Slightly delayed



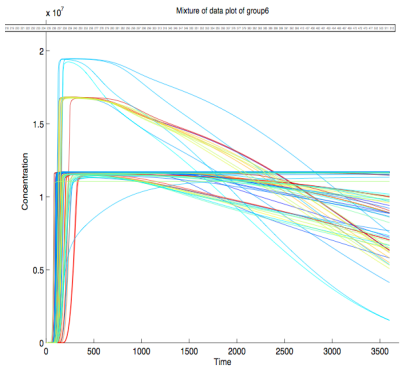
Cluster 4

Amplitude increased, Transient → sustained



Cluster 5

Duration enlarged, sustained

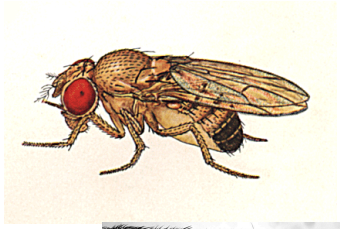


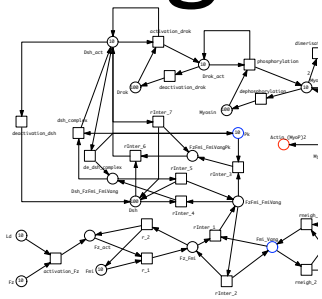
Cluster 6

- Raf, MEK, ERK generally, reduce the duration of the signalling

- Raf-phosphatase, MEK-phosphatase, ERK-phosphatase generally, convert ERK activation from transient to sustained.

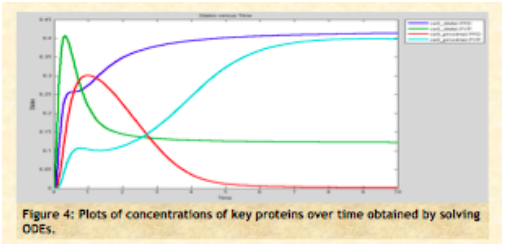
Multiscale from signalling to organs



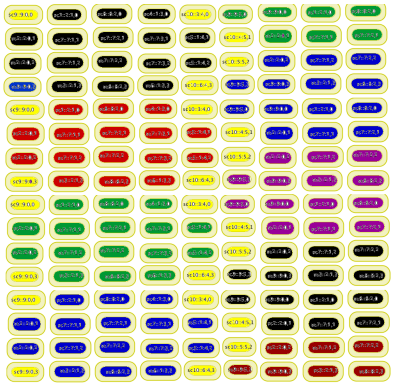


Petri nets (*coloured, hierarchical*)

Monika Heiner



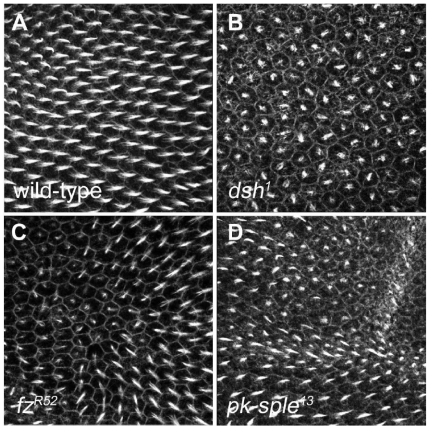
ODEs, stochastics

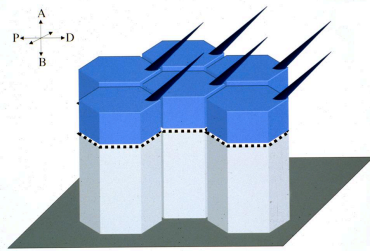


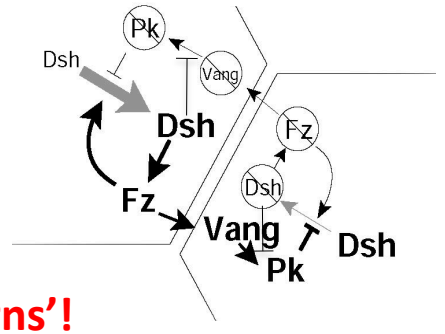
P-systems

(InfoBiotics - Nottingham)

Planar Cell Polarity







Pam Gao, David Tree

→ **Design & genetically engineer 'patterns'!**

BioModel Engineering

- Takes place at the interface of computing science, mathematics, engineering and biology.
- A systematic approach for **designing**, **constructing** and **analyzing** computational models of biological systems.
- Some inspiration from efficient software engineering strategies.
- Not engineering biological systems *per se,* but
  - describes their structure and behavior,
  - in particular at the level of intracellular molecular processes,
  - using computational tools and techniques in a principled way.

Rainer Breitling, David Gilbert, Monika Heiner, Richard Orton (2008). A structured approach for the engineering of biochemical network models, illustrated for signalling pathways. Briefings in Bioinformatics

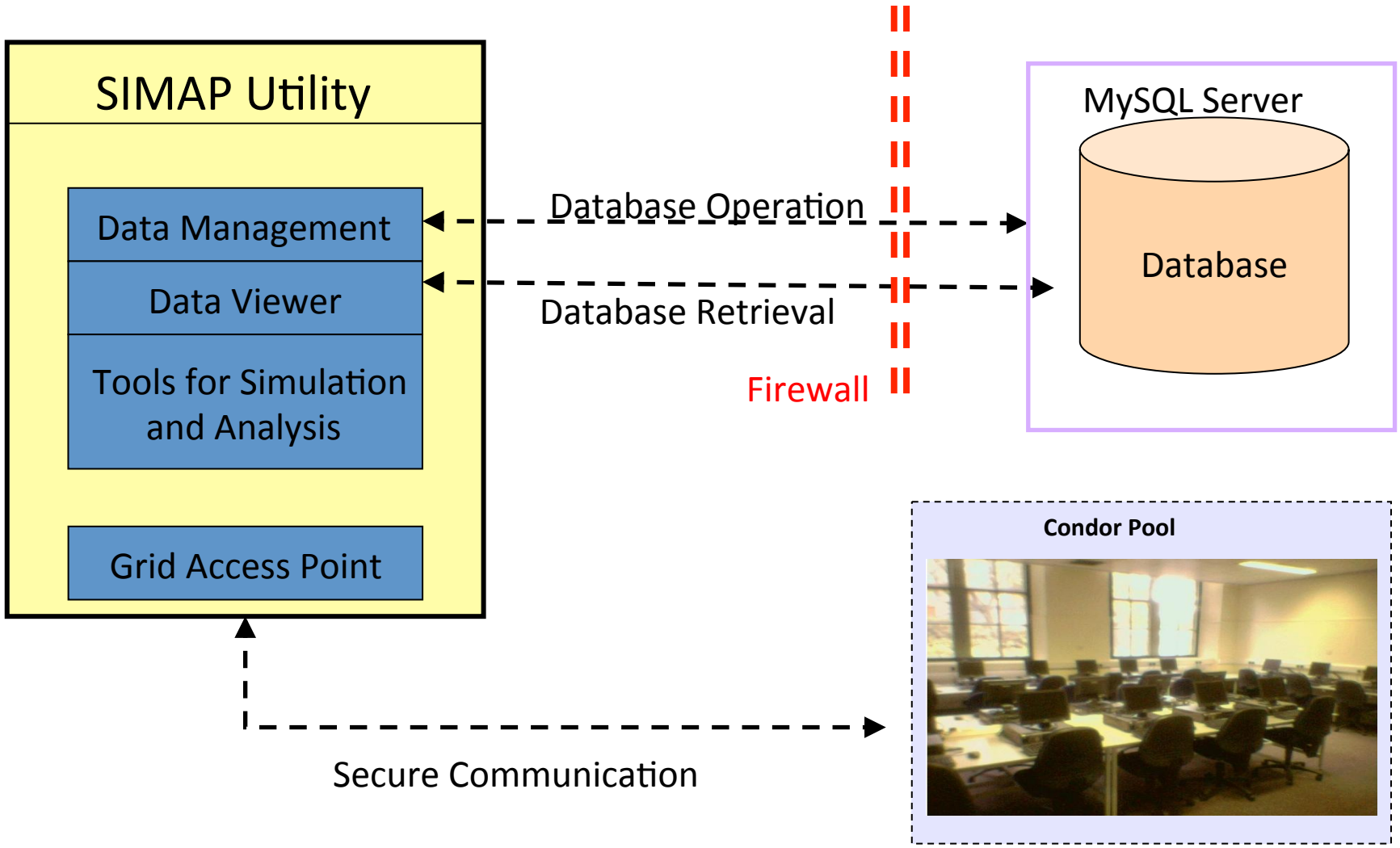
David Gilbert, Rainer Breitling, Monika Heiner, and Robin Donaldson (2009). An introduction to BioModel Engineering, illustrated for signal transduction pathways, 9th International Workshop, WMC 2008, Edinburgh, UK LNCS Volume 539, pp13-28

Rainer Breitling, Robin Donaldson, David Gilbert, Monika Heiner (2010): Biomodel Engineering - From Structure to Behavior; : Trans. Comp Systems Biology XII, Springer LNBI 5945, pp. 1-12

SIMAP Utility – BioModel engineering platform

- **Intuitive UI interface**
- **Model creation and editing**
- **Model simulation**
- **Embedded analytical tools**
  - Parameter scanning (multi-core/threaded/Grid-enabled)
  - Sensitivity analysis
  - Model fitting (genetic algorithm)
  - Advanced model checking (MC2)
- **Database integration and management**
- **Database of models, biochemical & patient data**
- **Model concurrent version system**
- **Gene Knockdown *in-silico***
- **Grid enabled**

SIMAP Utility



Formal Methods in Molecular Biology

- Dagstuhl Seminar
- February 2009
- Modelling competition…

•Transactions on Computational Systems Biology XII:
Special Issue on Modeling Methodologies. Springer LNBI 5945
Priami, Breitling, Gilbert, Heiner, Uhrmacher (Eds.) (2010)

• April 2011 - (http://www.dagstuhl.de/11151)

Subtext

- Can modelling ever be useful?
  - Explanations
  - Predictions
- What did it tell us? NFA → drug targeting
- When should we invest in modelling? ….

Acknowledgements

DTI Beacon Project Biological Pathway Simulator and Analyser

Glasgow:
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Multiple knockdowns: *Pam Gao, Robin Donaldson, Nikolaus Machuy (MP Berlin)*

BioNessie Utility: *Xuan Liu, Jun Wang*

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