A structured approach … Part III Biological applications

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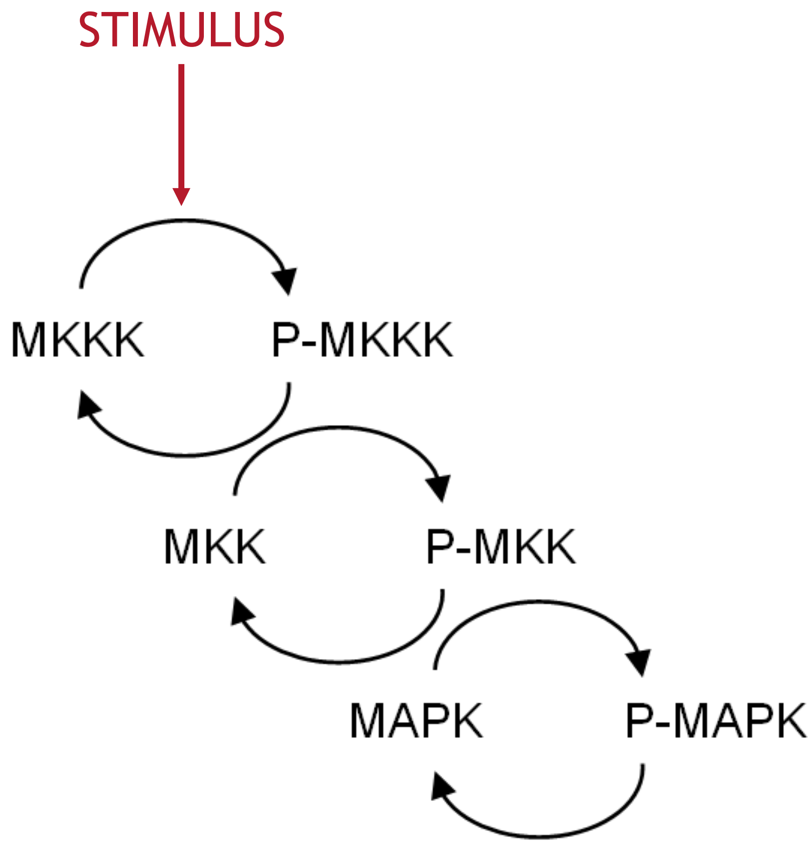
MAPK Pathway

- 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,

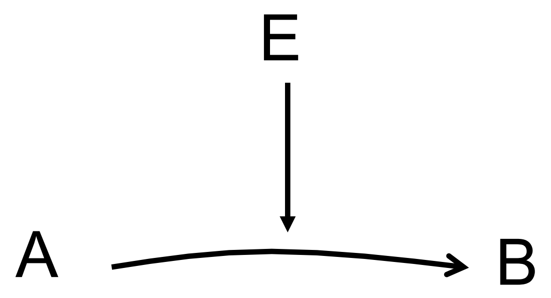
- Represents an important drug target.

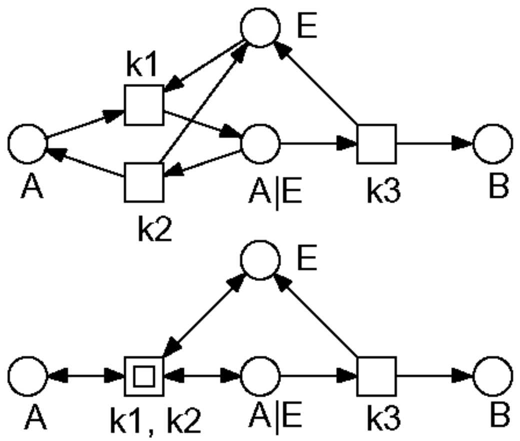


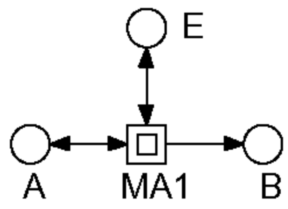
MA1: Mass action for enzymatic reaction - phosphorylation

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

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







Differential equations

Enzymatic reaction

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

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

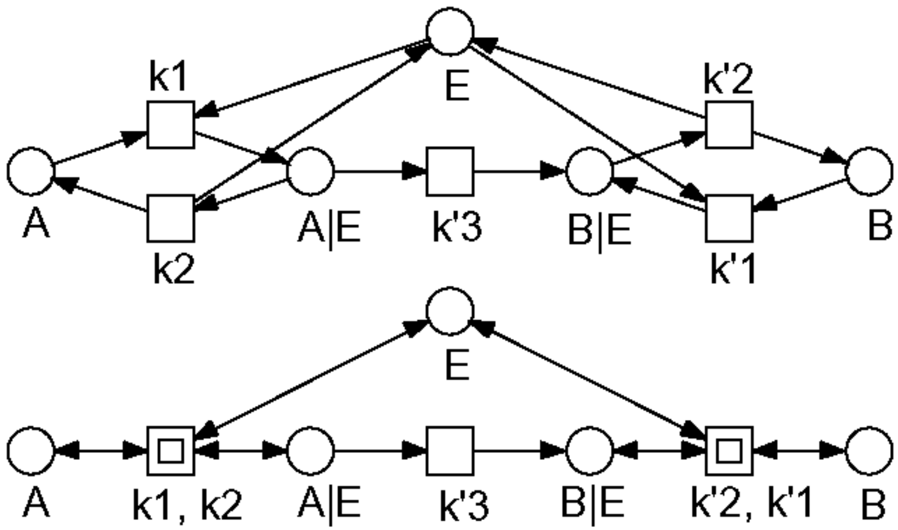
$$\frac{d[A \mid E]}{dt} = +k_1 \times [A] \times [E] - k_2 \times [A \mid E] - k_3 \times [A \mid E]$$

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

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

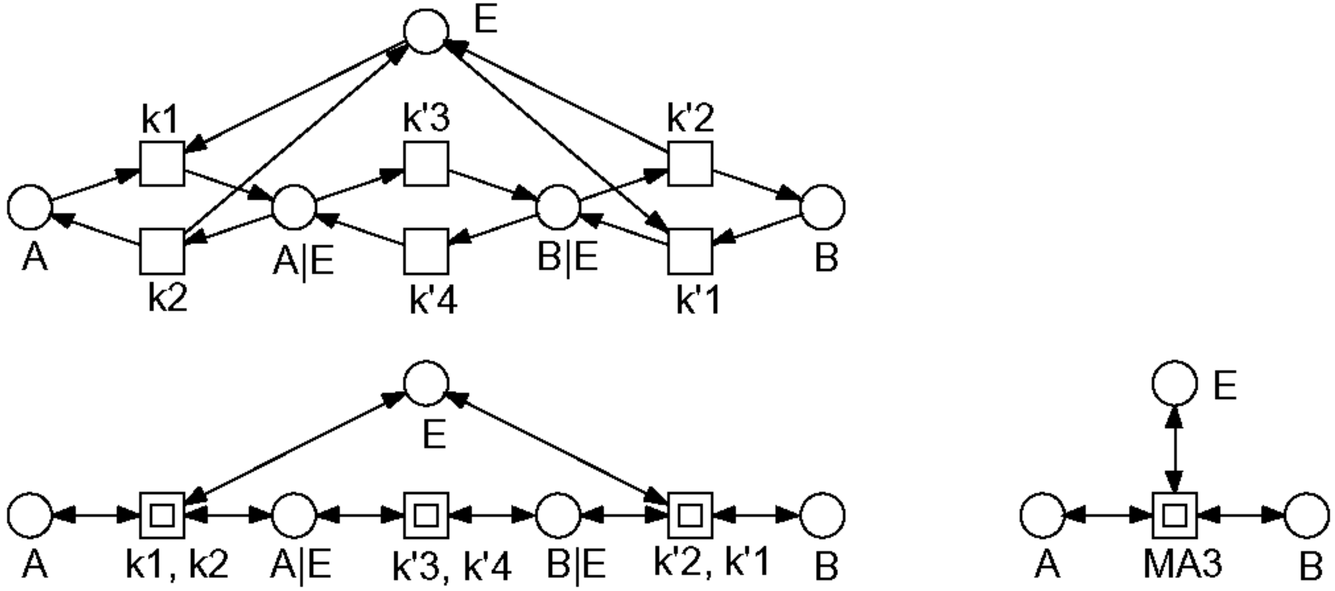
MA2 model

$$A + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} A \mid E \xrightarrow{k_3} B \mid E \underset{k5}{\overset{k_4}{\rightleftarrows}} B + E$$



MA3 model

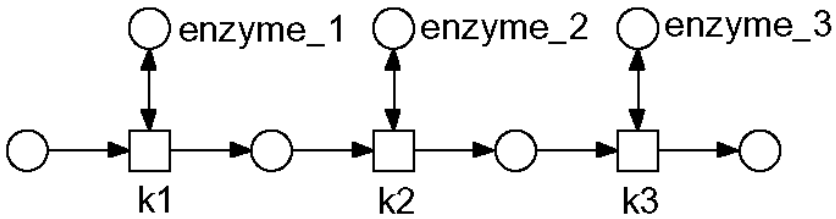
$$A + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} A \mid E \underset{k_6}{\overset{k_3}{\rightleftarrows}} B \mid E \underset{k\,5}{\overset{k_4}{\rightleftarrows}} B + E$$

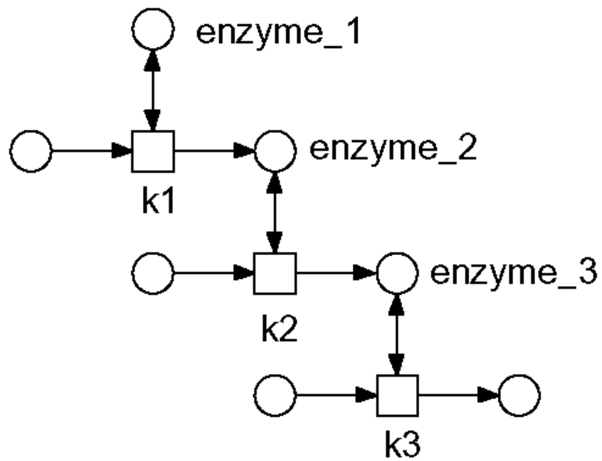


Multiple substrates

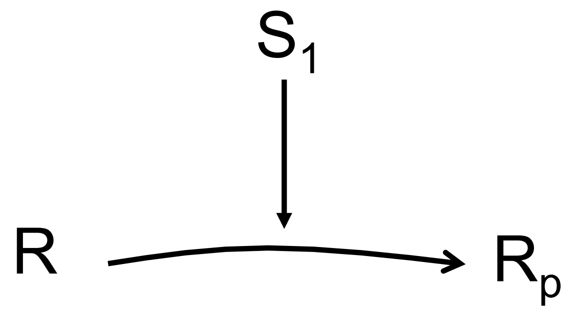
$$A_1 + A_2 + E \underset{k_{2_{A_1}}}{\overset{k_{1_{A_1}}}{\rightleftarrows}} A_1|E + A_2 \underset{k_{2_{A_2}}}{\overset{k_{1_{A_2}}}{\rightleftarrows}} A_1|A_2|E \xrightarrow{k_3} B_1 + B_2 + E$$

Metabolic pathways vs Signalling Pathways
(Petri Nets)





Mass action for enzymatic reaction - phosphorylation

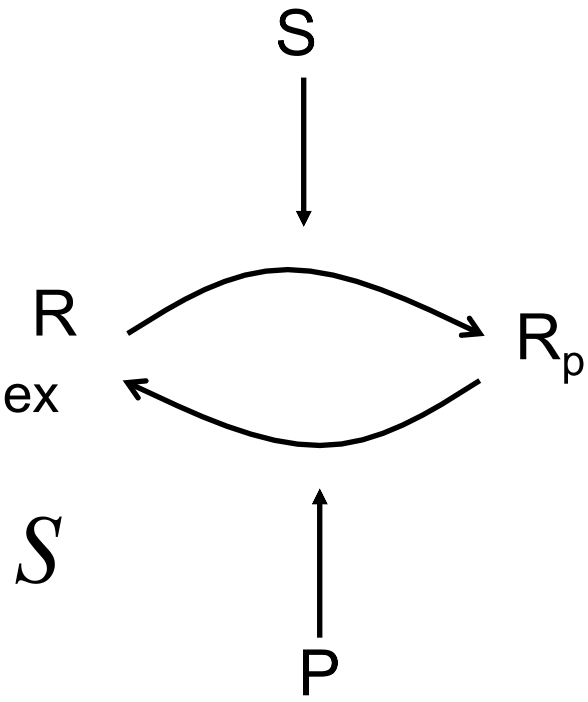


- R: substrate,
- R_p: product (phosphorylated R)
- S_1: enzyme (kinase)
- $R|S_1$ substrate-enzyme complex

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

Phosphorylation - dephosphorylation step
Mass action model 1

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

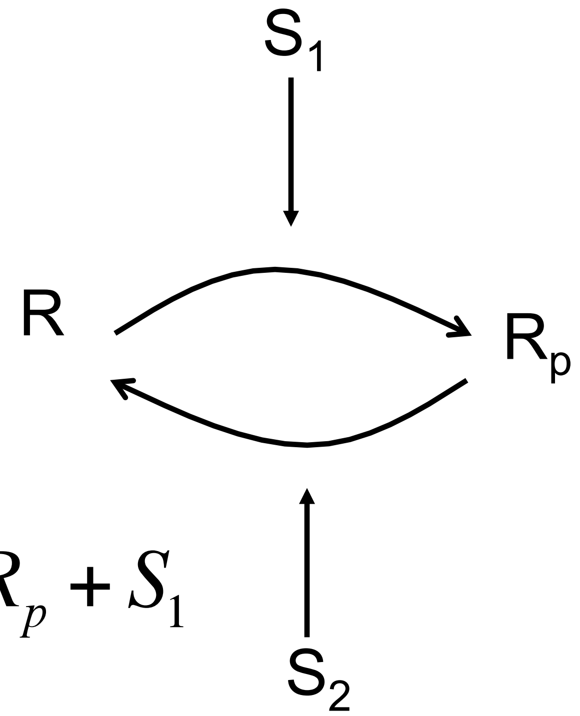


$$R+S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} R \mid S \xrightarrow{k_3} R_p + S$$

$$R+P \xleftarrow{kr_3} R_p \mid P \underset{kr_2}{\overset{kr_1}{\leftrightharpoons}} R_p + P$$

Phosphorylation - dephosphorylation loop Mass action model 2

- 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

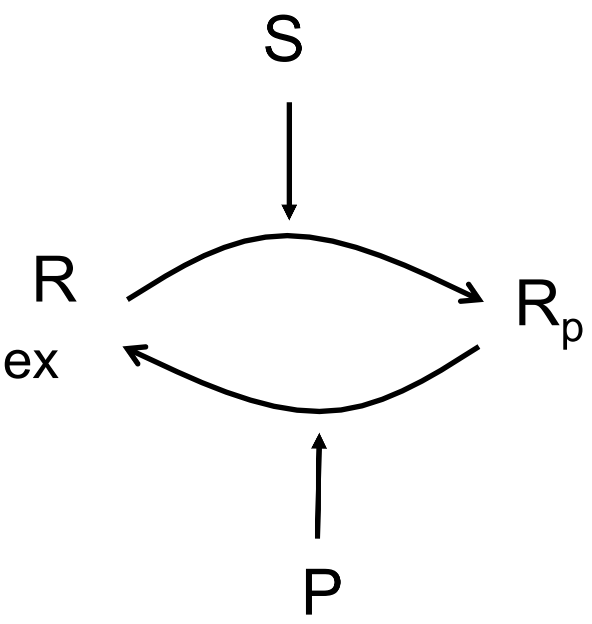


$$R + S_1 \overset{k_1}{\underset{k_2}{\rightleftarrows}} R \mid S_1 \xrightarrow{k_3} R_p \mid S_1 \overset{k_4}{\underset{k\,5}{\rightleftarrows}} R_p + S_1$$

$$R + S_2 \overset{kr_4}{\underset{kr\,5}{\leftrightarrows}} R \mid S_2 \xleftarrow{kr_3} R_p \mid S_2 \overset{kr_1}{\underset{kr_2}{\leftrightarrows}} R_p + S_2$$

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

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

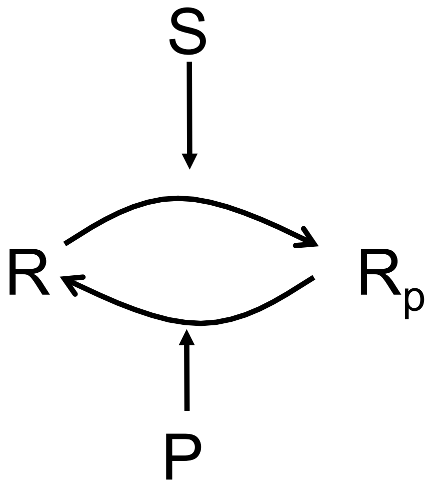


$$R+S \underset{k_2}{\overset{k_1}{\rightleftarrows}} R \mid S \underset{k_6}{\overset{k_3}{\rightleftarrows}} R_p \mid S \underset{k5}{\overset{k_4}{\rightleftarrows}} R_p + S$$

$$R+P \underset{kr_5}{\overset{kr_4}{\leftrightarrows}} R \mid P \underset{kr\,6}{\overset{kr_3}{\leftrightarrows}} R_p \mid P \underset{kr_2}{\overset{kr_1}{\leftrightarrows}} R_p + P$$

Michaelis-Menten equation for phosphorylation-dephosphorylation

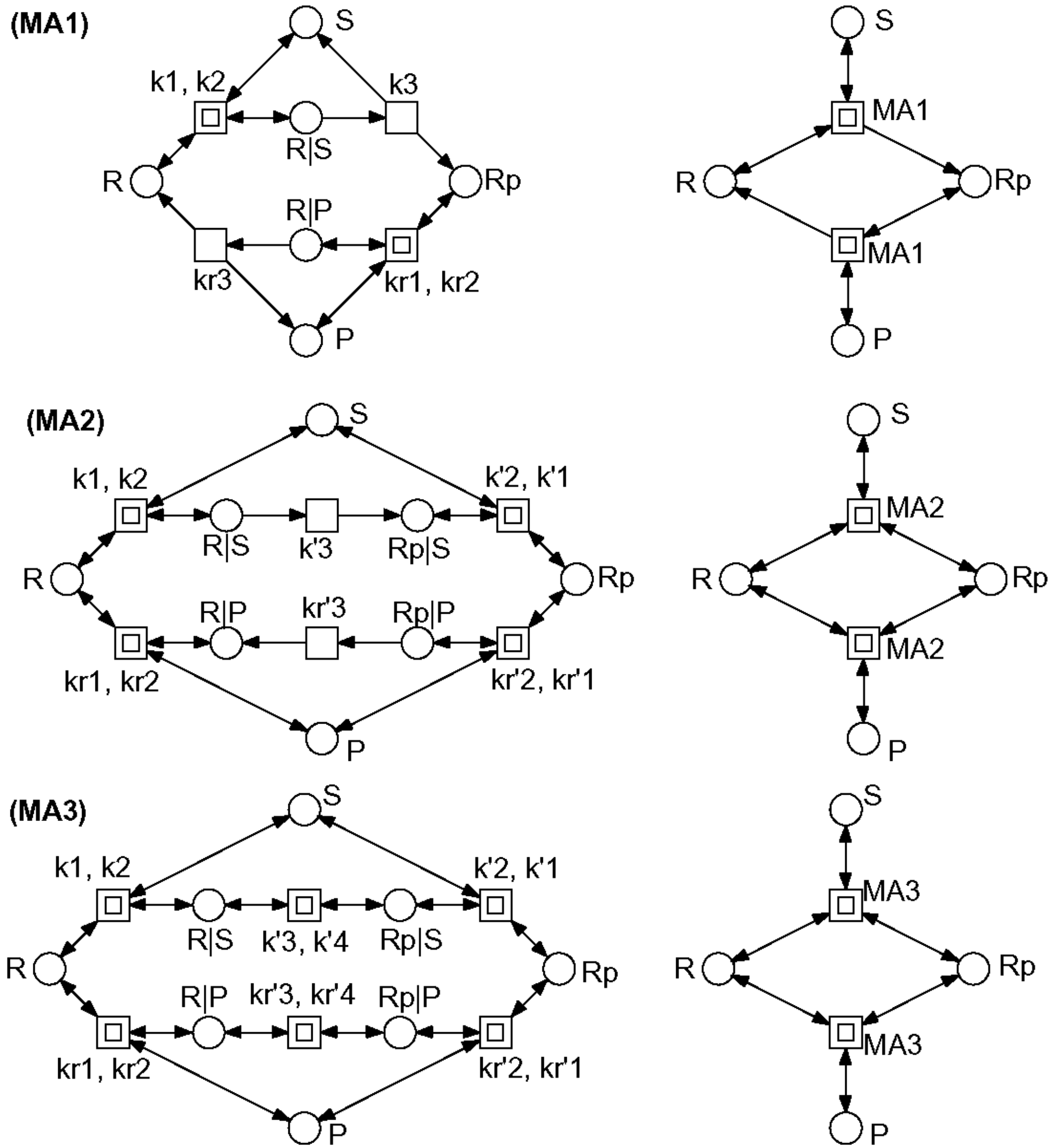
$$V = k_3 \times [S] \times \frac{[R]}{(K_{m1} + [R])} - k_3' \times \frac{[R_p]}{(K_{m2} + [R_p])}$$



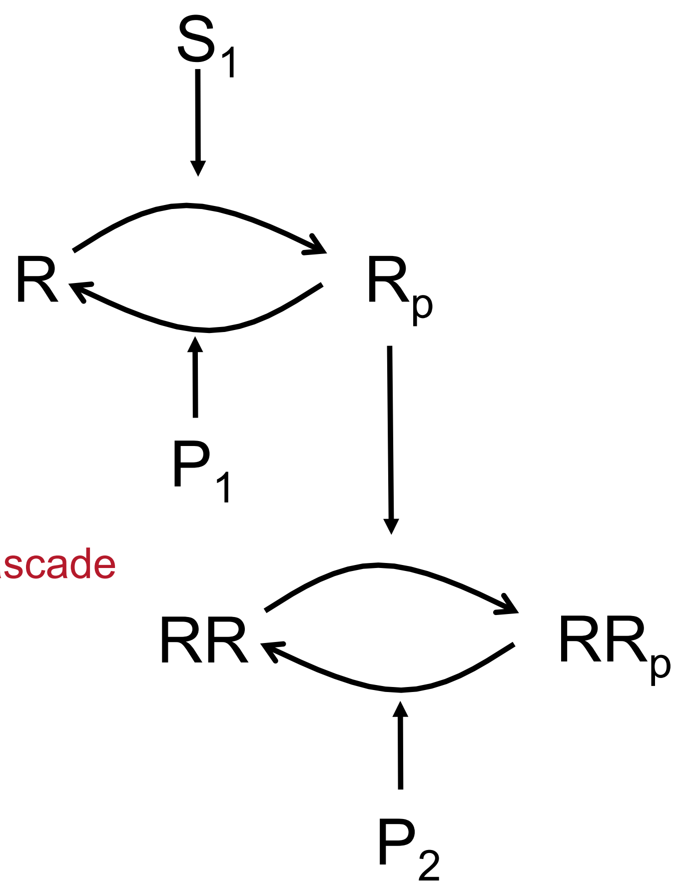
- Assumptions:
  1. No product reverts to initial substrate
  2. MM Equation holds at initial stage of reaction before concentration of product is appreciable
  3. [Enzyme] << [Substrate]
- K_m is [Substrate] at which the reaction rate is half its maximum value
- dR_p/dt == reaction rate V
- k_3 x S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

Questions

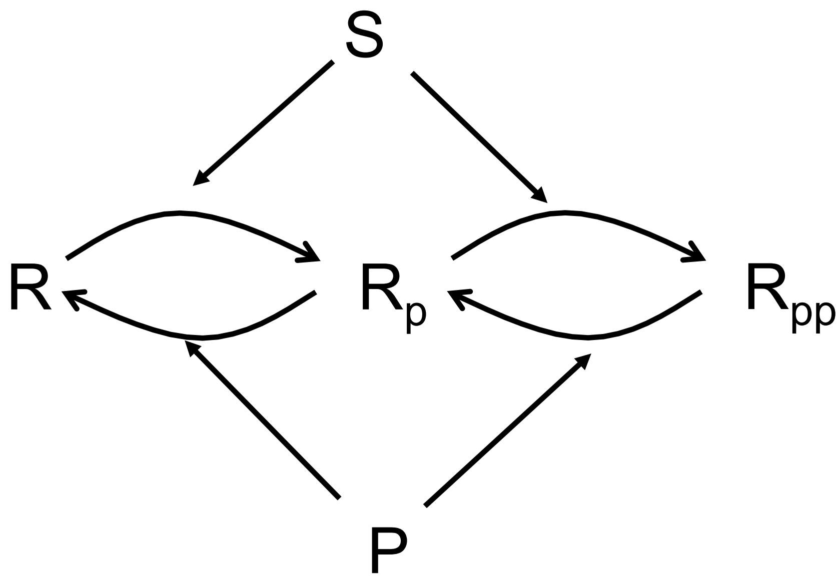
- Is Michaelis-Menten adequate for phosphorylation pathways?
- Is Mass Action sufficient/correct for these pathways?
- What is the effect of negative feedback?
- Can we confirm the ‘negative feedback amplifer’ behaviour in both MM and MA models
- Can oscillators be built?
- Overall, what are the rules for component-based construction?


(MA1)
S
k1, k2
k3
R|S
R
Rp
R|P
kr3
kr1, kr2
P
S
MA1
R
Rp
MA1
P
(MA2)
S
k1, k2
k'2, k'1
R|S
k'3
Rp|S
R
Rp
R|P
kr'3
Rp|P
kr1, kr2
kr'2, kr'1
P
S
MA2
R
Rp
MA2
P
(MA3)
S
k1, k2
k'2, k'1
R|S
k'3, k'4
Rp|S
R
Rp
R|P
kr'3, kr'4
Rp|P
kr1, kr2
kr'2, kr'1
P
S
MA3
R
Rp
MA3
P

Composition
Vertical & horizontal

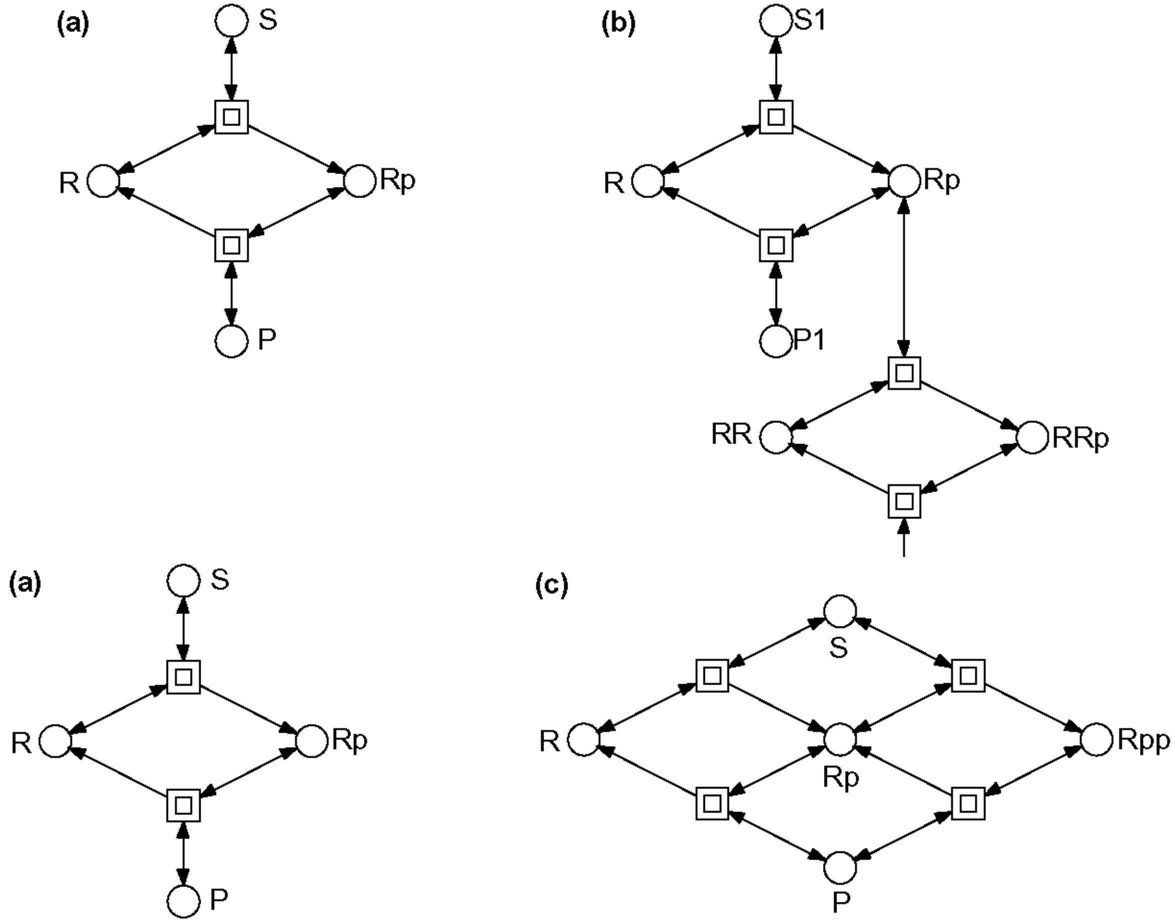


2-stage cascade

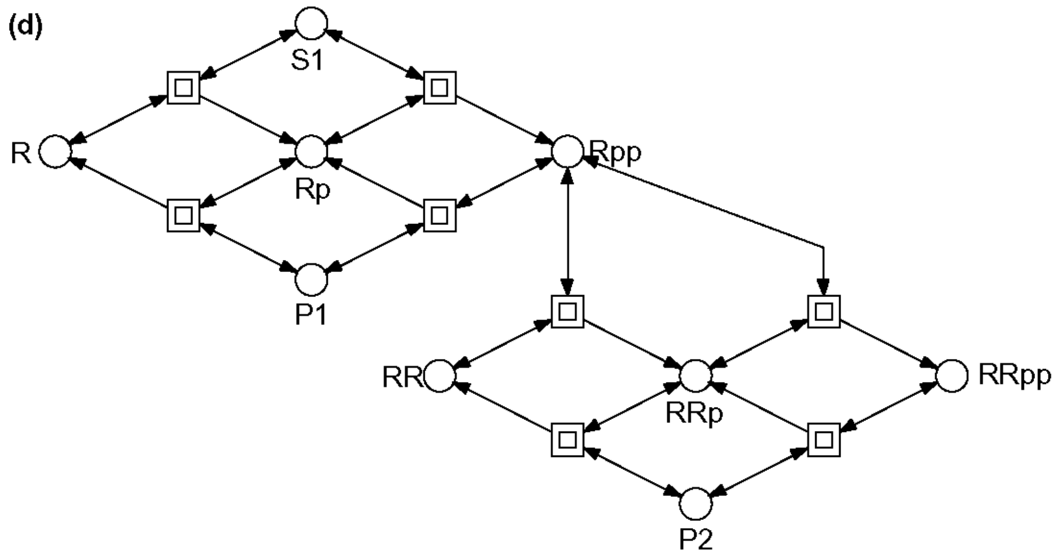


1-stage cascade
double phosphorylation step

Composition
Vertical & horizontal



Two stage, double phosphorylation



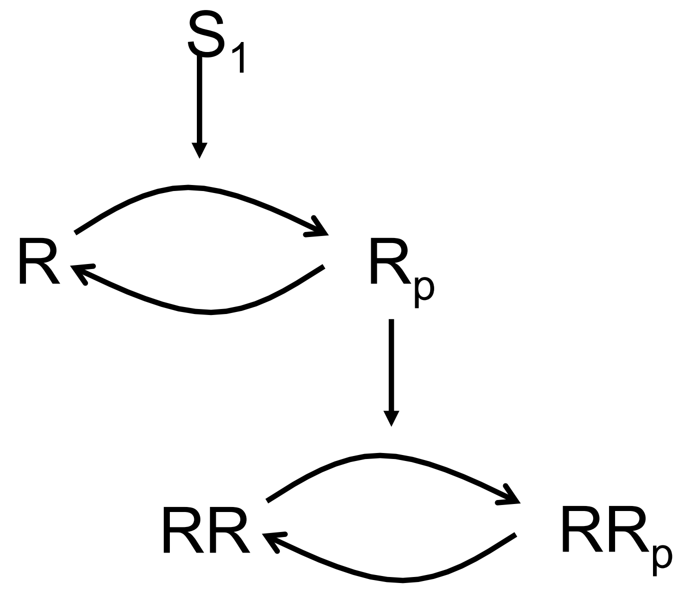
Phosphorylation cascade: 2-stage, Mass Action model 1

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

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

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

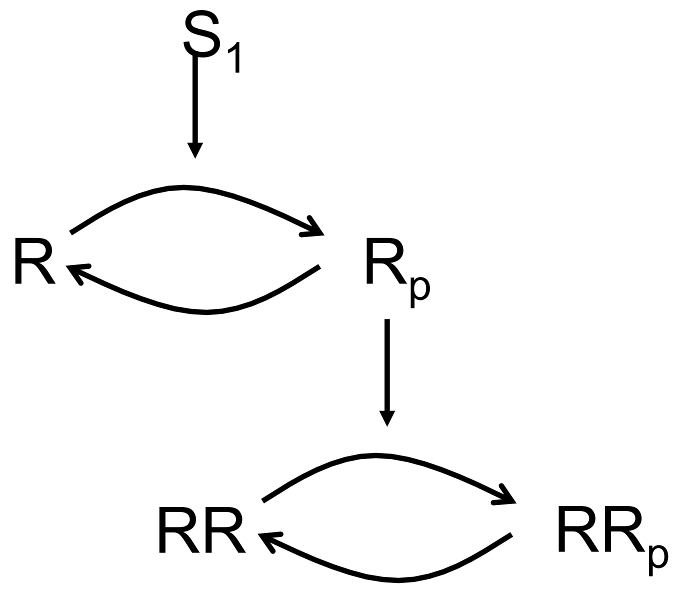
$$RR + P_2 \xleftarrow{kk_3'} RR \mid P_2 \underset{kk_2'}{\overset{kk_1'}{\rightleftharpoons}} RR_p + P_2$$



Phosphorylation cascade: 2-stage, Michaelis-Menten

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

$$\frac{dRR_p}{dt} = \frac{kk_3 \times R_p \times RR}{KK_{m1} + RR} - \frac{kk_3' \times RR_p}{KK_{m2} + RR_p}$$



3-stage Phosphorylation cascade (Mass Action)

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

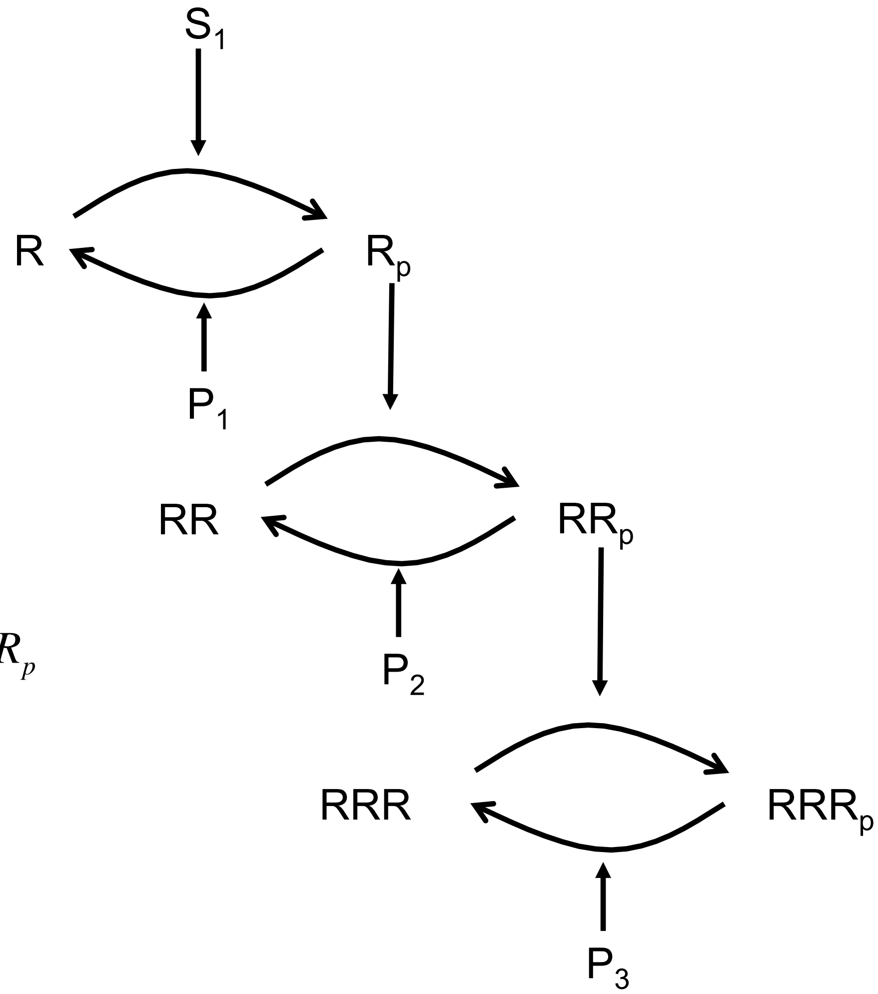
$$R+P_1 \xleftarrow{k_3'} R_p|P_1 \underset{k_2'}{\overset{k_1'}{\leftrightharpoons}} R_p + P_1$$

$$RR + R_p \underset{kk_2}{\overset{kk_1}{\rightleftharpoons}} RR|R_p \xrightarrow{kk_3} RR_p + R_p$$

$$RR+ P_2 \xleftarrow{kk_3'} RR_p|P_2 \underset{kk_2'}{\overset{kk_1'}{\leftrightharpoons}} RR_p + P_2$$

$$RRR + RR_p \underset{kkk_2}{\overset{kkk_1}{\rightleftharpoons}} RRR|RR_p \xrightarrow{kkk_3} RRR_p + RR_p$$

$$RRR+ P_3 \xleftarrow{kkk_3'} RRR_p|P_3 \underset{kkk_2'}{\overset{kkk_1'}{\leftrightharpoons}} RRR_p + P_3$$

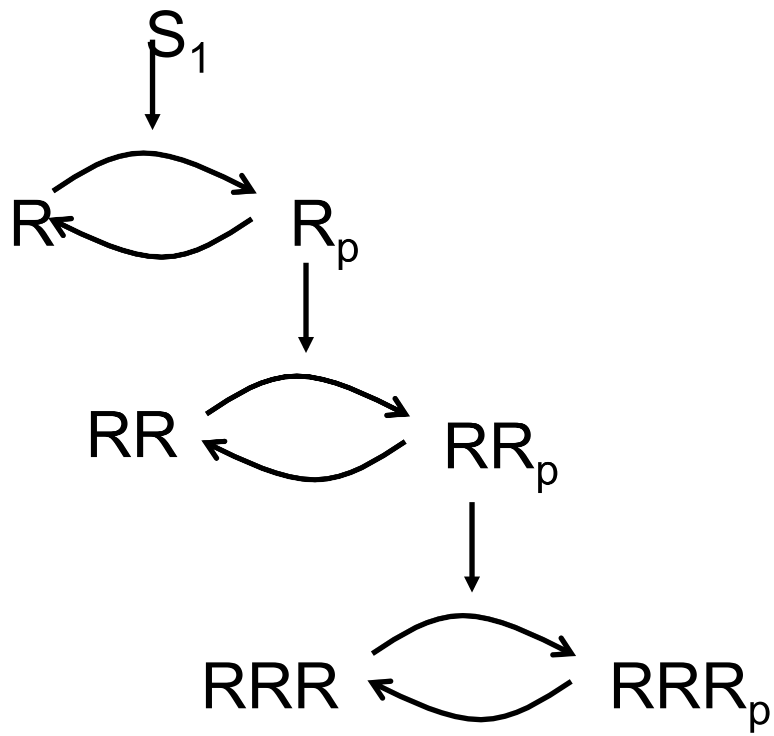


Phosphorylation cascade: 3-stage, Michaelis-Menten

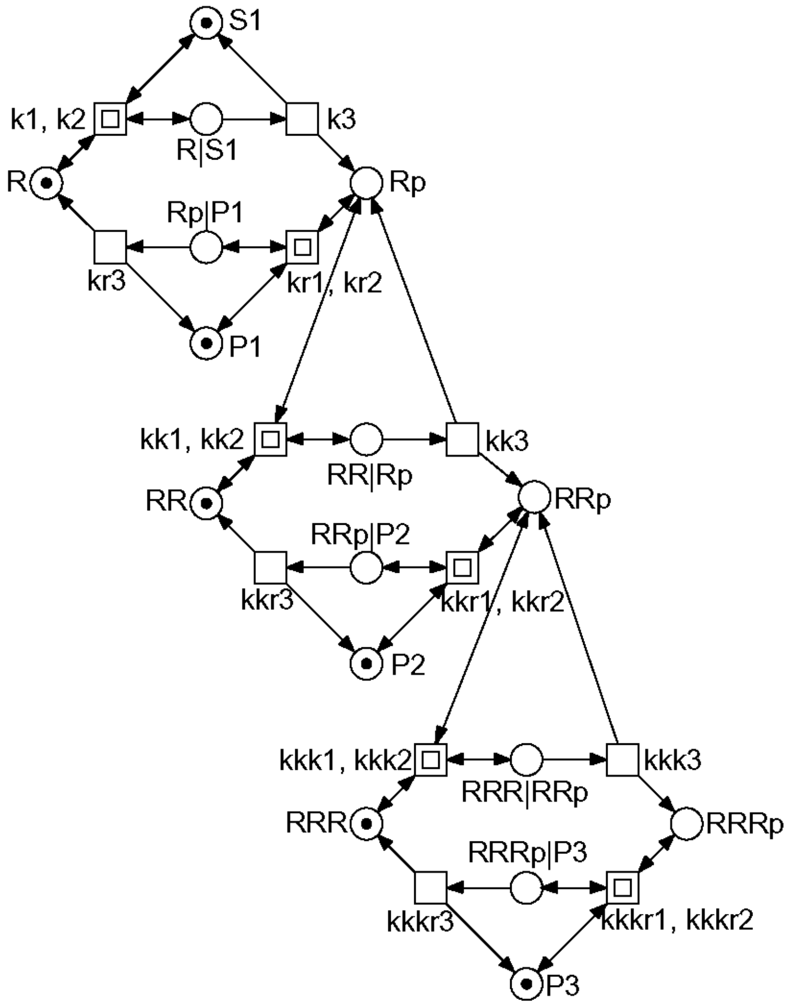
$$\frac{dR_p}{dt} = \frac{k_3 \times S_1 \times R}{K_{m1} + R} - \frac{k_3' \times R_p}{K_{m2} + R_p}$$

$$\frac{dRR_p}{dt} = \frac{kk_3 \times R_p \times RR}{KK_{m1} + RR} - \frac{kk_3' \times RR_p}{KK_{m2} + RR_p}$$

$$\frac{dRRR_p}{dt} = \frac{kkk_3 \times RR_p \times RRR}{KKK_{m1} + RRR} - \frac{kkk_3' \times RRR_p}{KKK_{m2} + RRR_p}$$

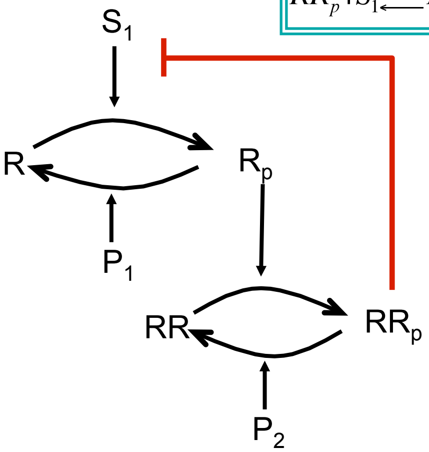


3-stage


S1
k1, k2
R|S1
k3
R
Rp
Rp|P1
kr3
kr1, kr2
P1
kk1, kk2
RR|Rp
kk3
RR
RRp
RRp|P2
kkr3
kkr1, kkr2
P2
kkk1, kkk2
RRR|RRp
kkk3
RRR
RRRp
RRRp|P3
kkkr3
kkkr1, kkkr2
P3

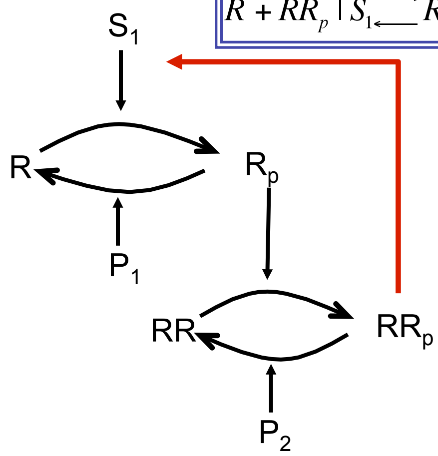
Phosphorylation cascade + feedback

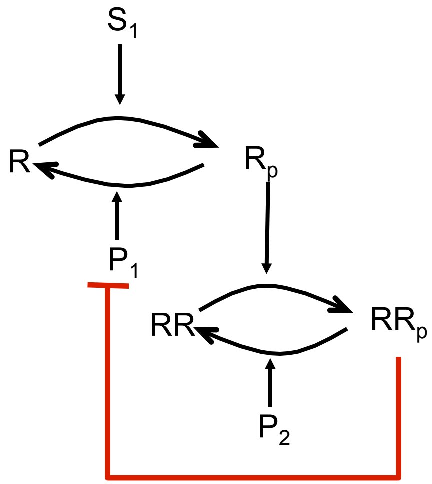
$$RR_p + S_1 \rightleftarrows RR_p \mid S_1$$



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

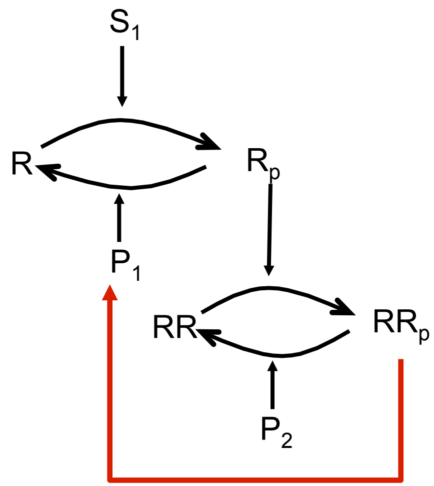
$$R + RR_p \mid S_1 \rightleftarrows R \mid RR_p \mid S_1 \longrightarrow RR_p \mid S_1$$



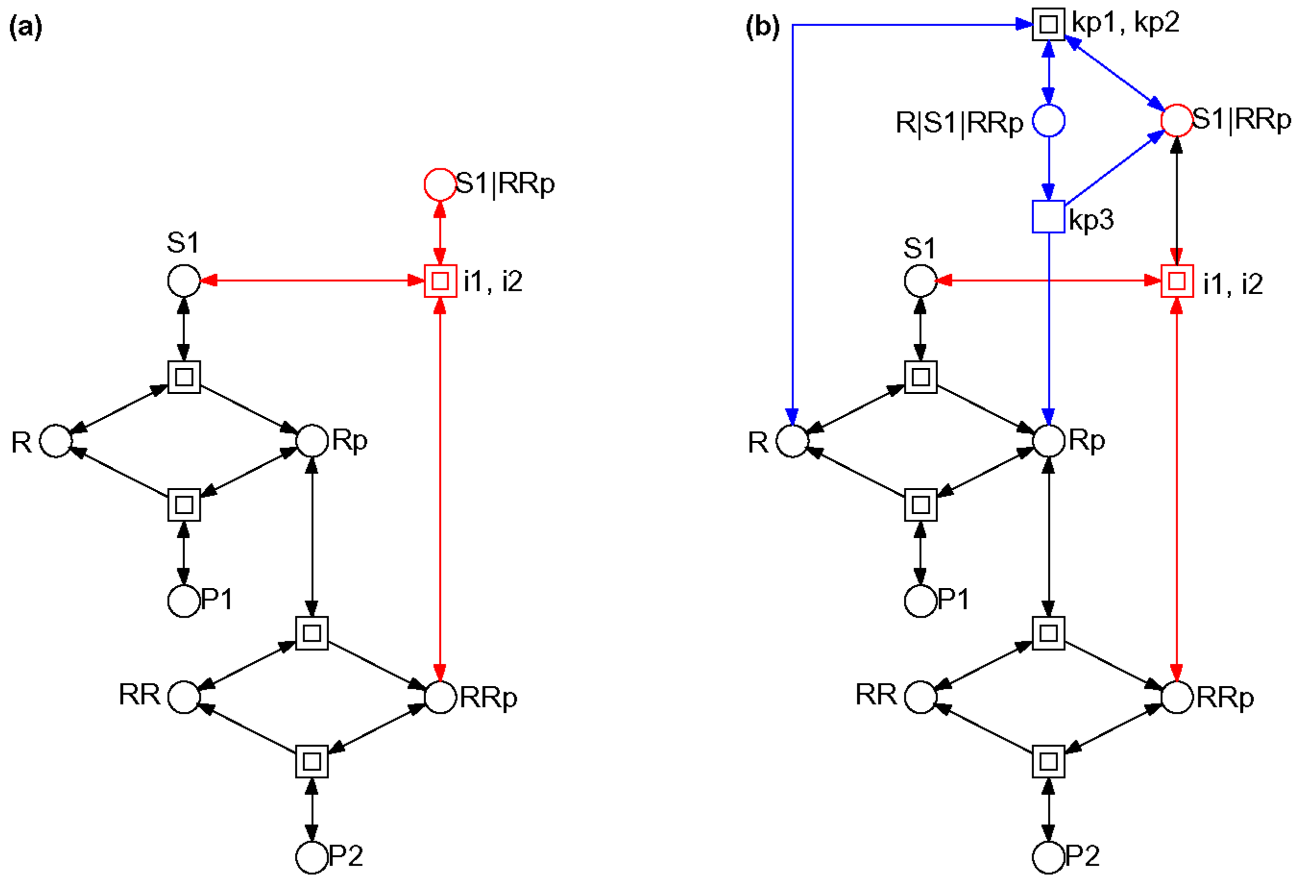


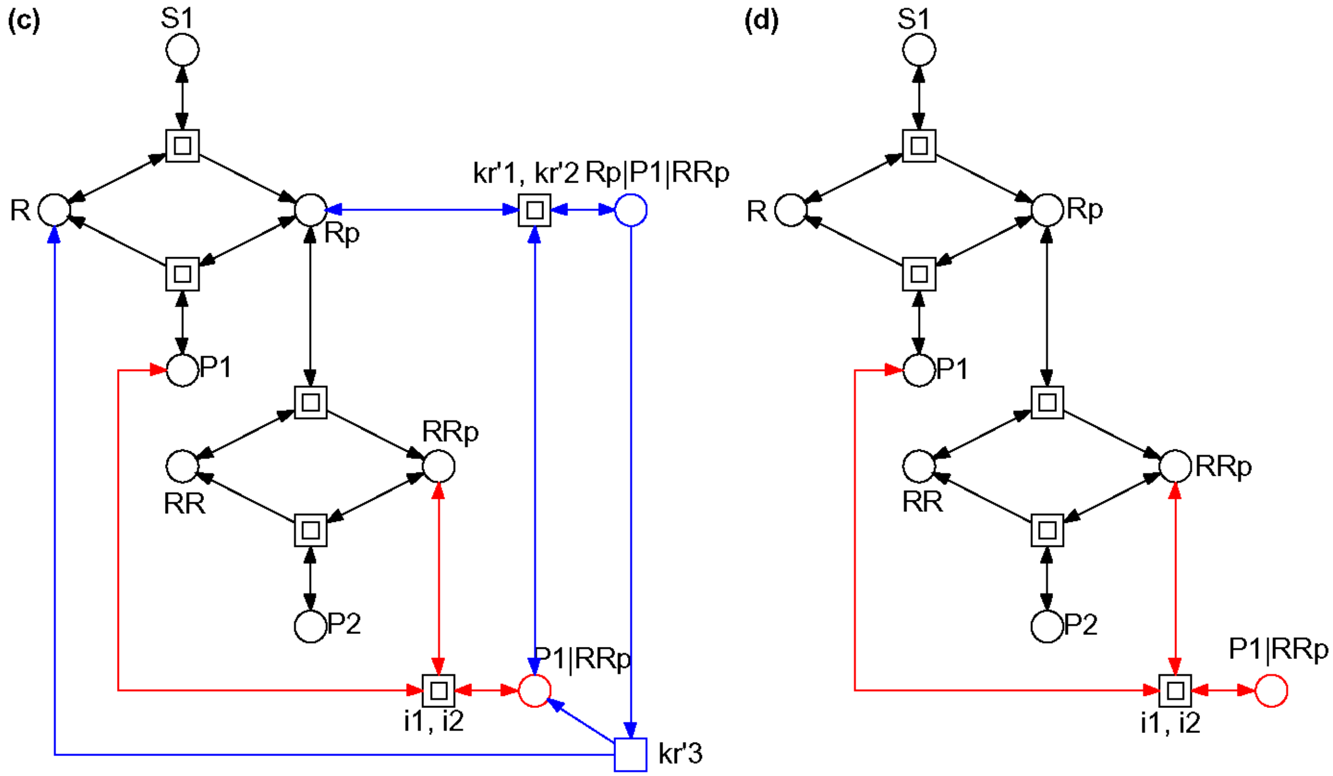
$$RR_p + P_1 \rightleftarrows RR_p \mid P_1$$

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



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


(a)
S1|RRp
S1
i1, i2
R
Rp
P1
RR
RRp
P2
(b)
kp1, kp2
R|S1|RRp
S1|RRp
kp3
S1
i1, i2
R
Rp
P1
RR
RRp
P2


(c)
S1
R
Rp
kr'1, kr'2
Rp|P1|RRp
P1
RRp
RR
P2
P1|RRp
i1, i2
kr'3
(d)
S1
R
Rp
P1
RRp
RR
P2
P1|RRp
i1, i2

Phosphorylation cascade + negative feedback: 2-stage, Mass Action model 1

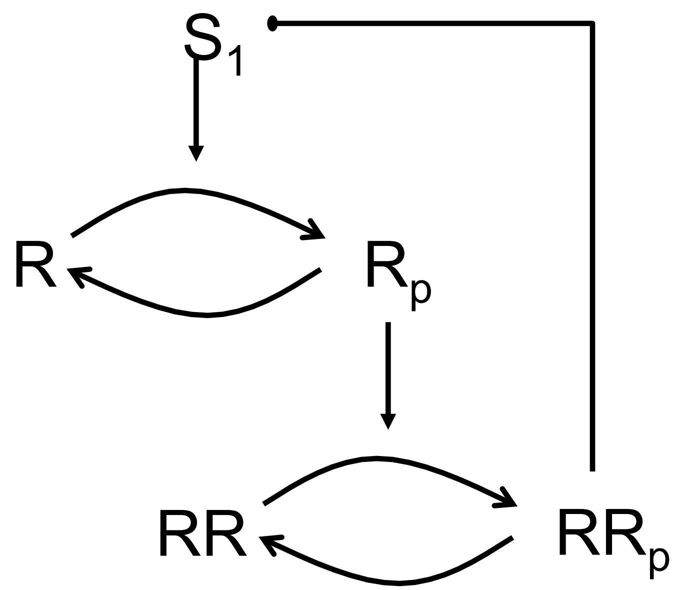
$$RR_p + S_1 \underset{ki'}{\overset{ki}{\rightleftharpoons}} RR_p \mid S_1$$

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

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

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

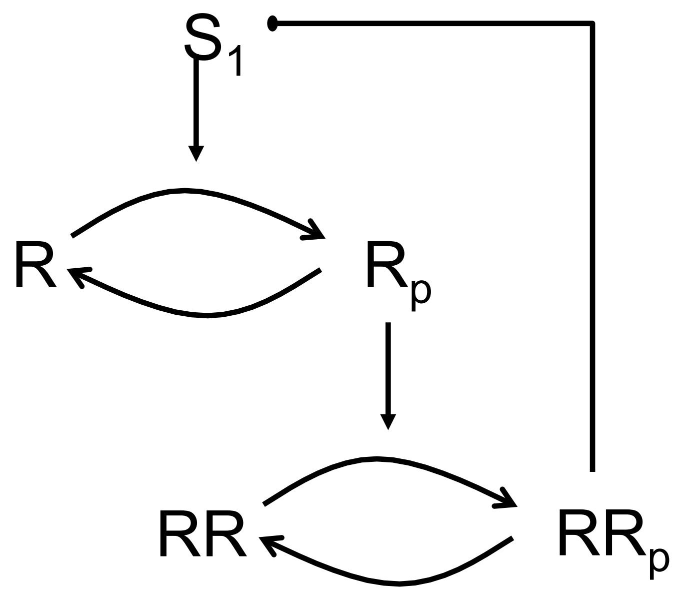
$$RR + P_2 \xleftarrow{kk_3'} RR \mid P_2 \underset{kk_2'}{\overset{kk_1'}{\leftrightharpoons}} RR_p + P_2$$



Phosphorylation cascade + negative feedback: 2-stage, Michaelis-Menten

$$\frac{dR_p}{dt} = \frac{k_3 \times S_1 \times R}{K_{m1} \times \left(1 + \frac{RR_p}{K_i}\right) + R} - \frac{k_3' \times R_p}{K_{m2} + R_p}$$

$$\frac{dRR_p}{dt} = \frac{kk_3 \times R_p \times RR}{KK_{m1} + RR} - \frac{kk_3' \times RR_p}{KK_{m2} + RR_p}$$



- Using Competitive Inhibition

$$V = V_{\max} \times \frac{[S]}{[S] + K_m \times \left(1 + \frac{[I]}{[K_i]}\right)}$$

- Ki is the dissociation constant for the SI complex

Phosphorylation cascade + negative feedback: 3-stage, Mass Action, model 1

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

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

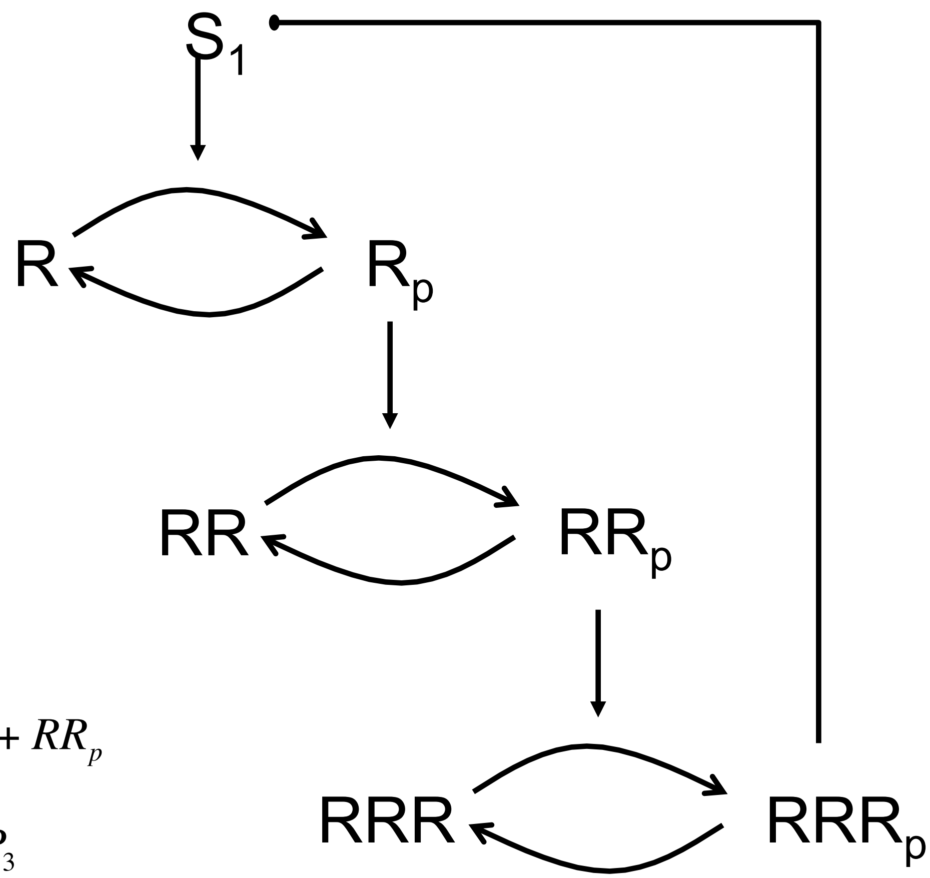
$$R + P_1 \xleftarrow{k_3'} R_p \mid P_1 \underset{k_2'}{\overset{k_1'}{\rightleftharpoons}} R_p + P_1$$

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

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

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

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

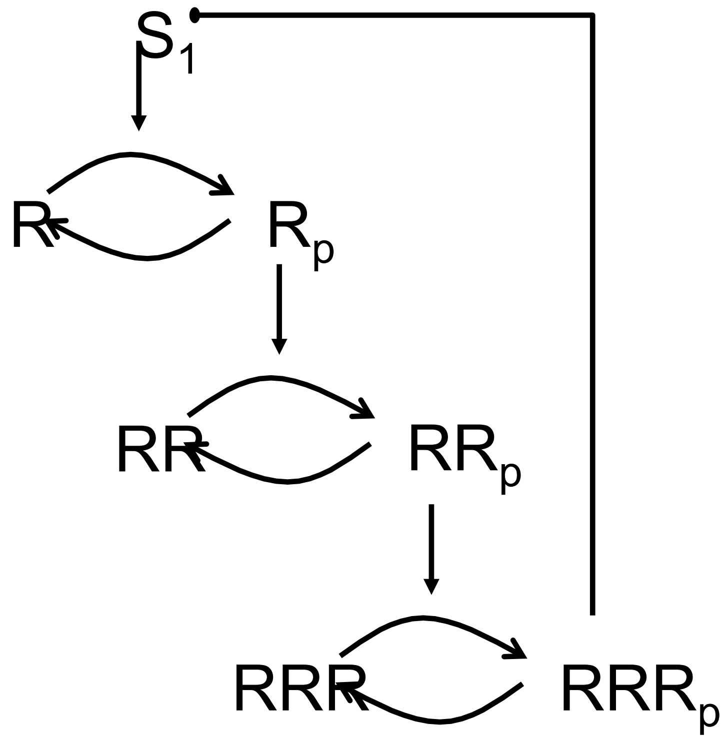


Phosphorylation cascade + negative feedback: 3-stage, Michaelis-Menten

$$\frac{dR_p}{dt} = \frac{k_3 \times S_1 \times R}{K_{m1} \times \left(1 + \frac{RRR_p}{K_i}\right) + R} - \frac{k_3' \times R_p}{K_{m2} + R_p}$$

$$\frac{dRR_p}{dt} = \frac{kk_3 \times R_p \times RR}{KK_{m1} + RR} - \frac{kk_3' \times RR_p}{KK_{m2} + RR_p}$$

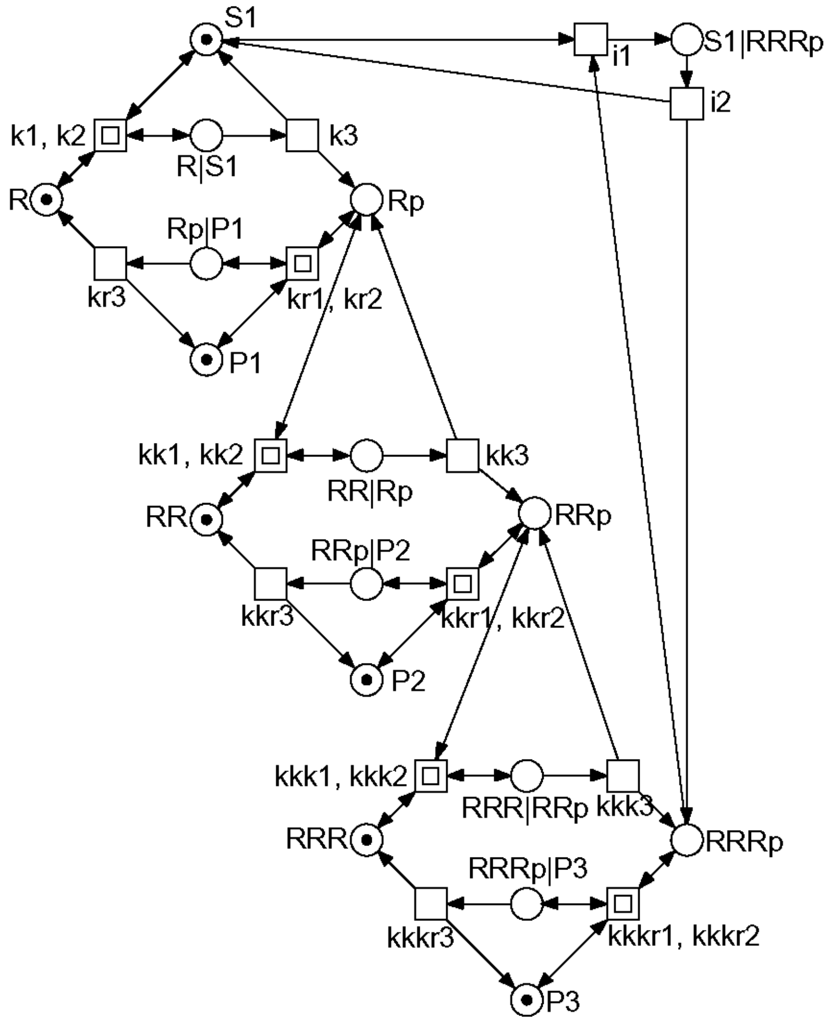
$$\frac{dRRR_p}{dt} = \frac{kkk_3 \times RR_p \times RRR}{KKK_{m1} + RRR} - \frac{kkk_3' \times RRR_p}{KKK_{m2} + RRR_p}$$



$$V = V_{max} \times \frac{[S]}{[S] + K_m \times \left(1 + \frac{[I]}{[K_i]}\right)}$$

- Using Competitive Inhibition
- Ki is the dissociation constant for the SI complex

3-stage, negative feedback



Phosphorylation cascade + negative feedback: 3-stage, Inhibitor on 2nd stage, Mass Action

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

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

$$R + P_1 \xleftarrow{k_3'} R_p|P_1 \underset{k_2'}{\overset{k_1'}{\leftrightarrows}} R_p + P_1$$

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

$$RR + P_2 \xleftarrow{kk_3'} RR_p|P_2 \underset{kk_2'}{\overset{kk_1'}{\leftrightarrows}} RR_p + P_2$$

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

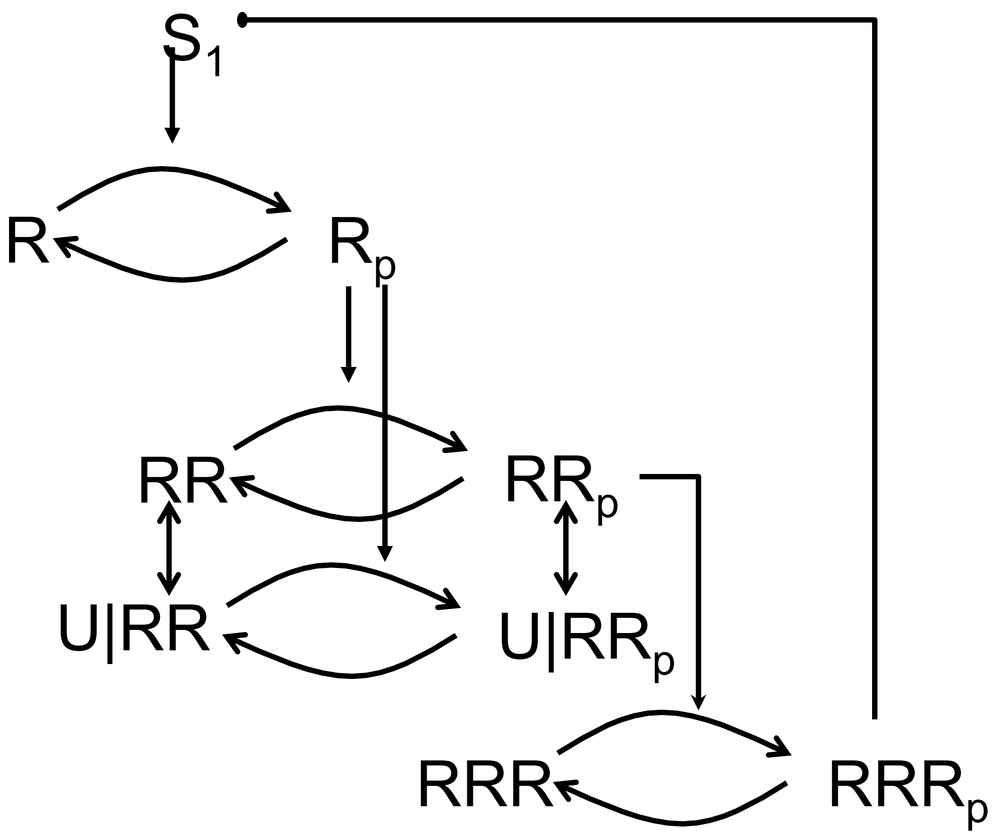
$$RRR + P_3 \xleftarrow{kkk_3'} RRR_p|P_3 \underset{kkk_2'}{\overset{kkk_1'}{\leftrightarrows}} RRR_p + P_3$$

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

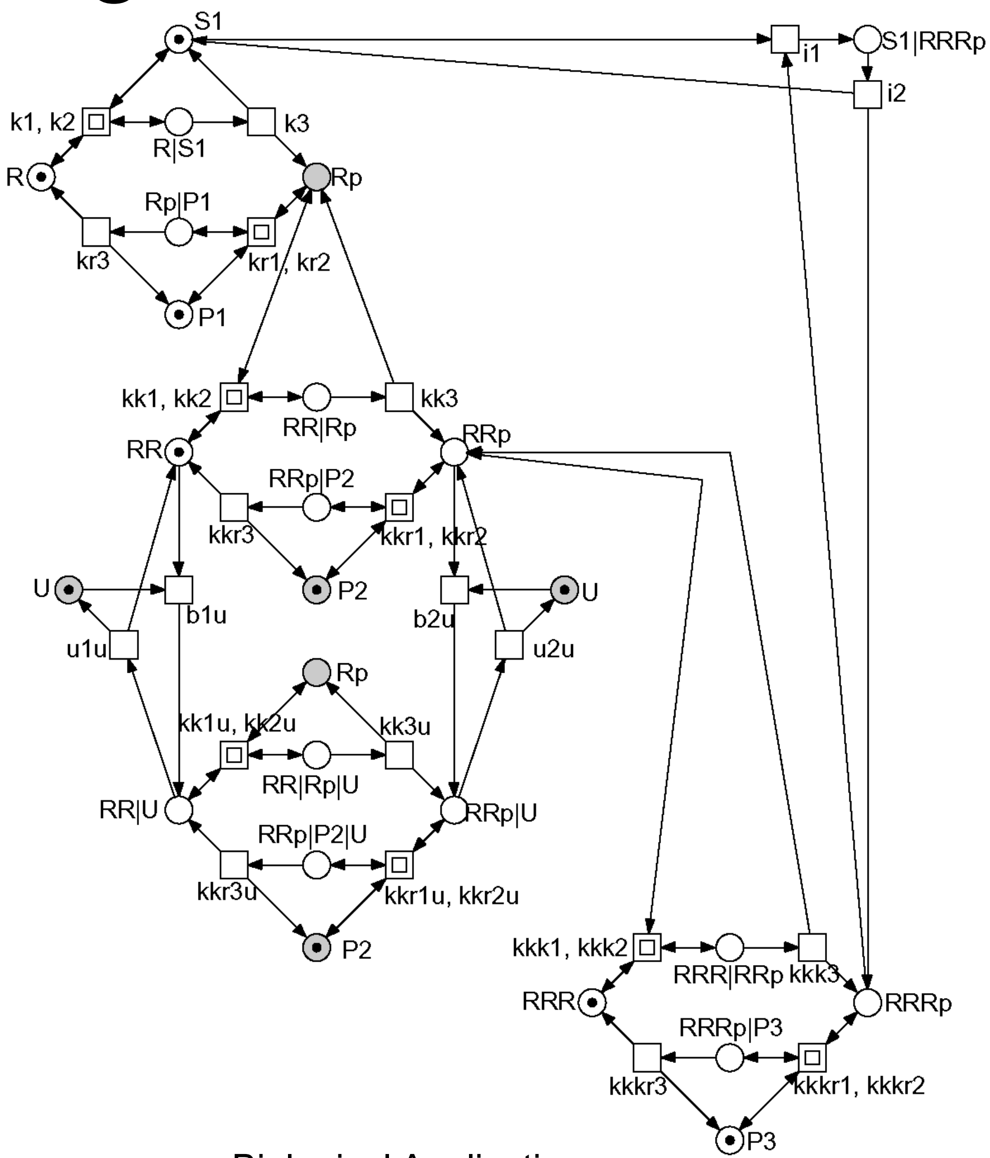
$$U + RR_p \underset{ku_2}{\overset{ku_1}{\rightleftarrows}} U|RR_p$$

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

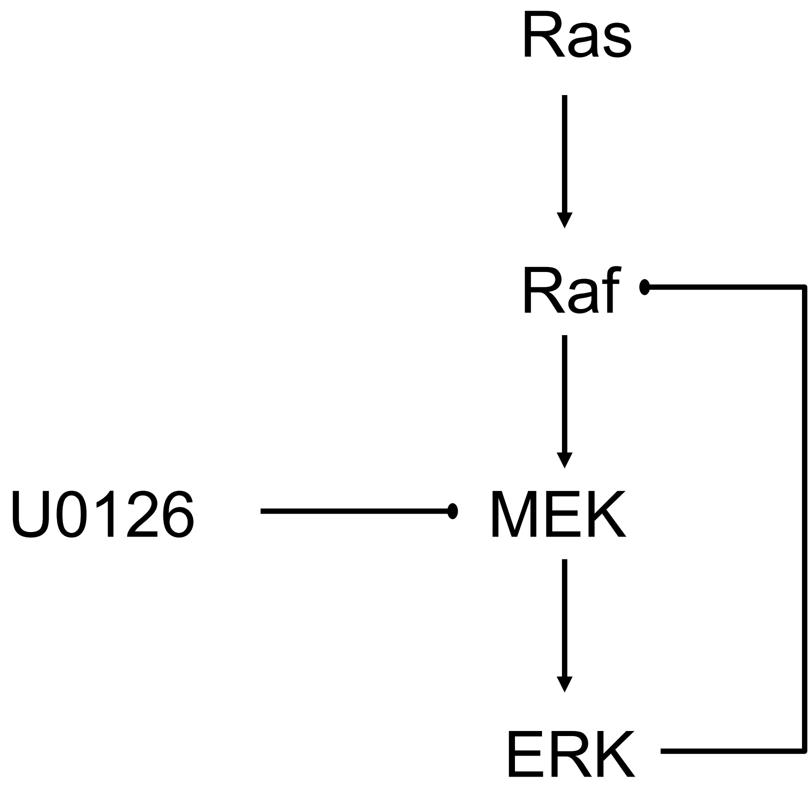
$$U|RR + P_2 \xleftarrow{kk_3'} U|RR_p|P_2 \underset{kk_2'}{\overset{kk_1'}{\leftrightarrows}} U|RR_p + P_2$$



3-stage, negative feedback + inhibitor



‘Real cascade & feedback’



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.

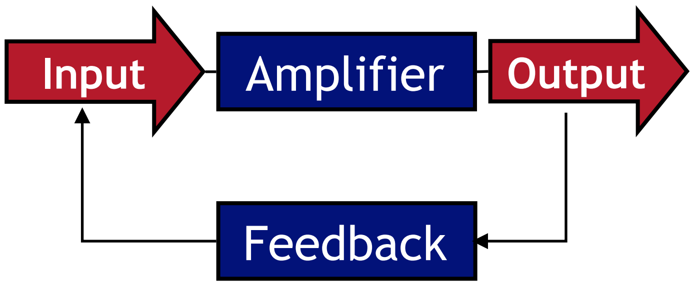
Negative Feedback Amplifier

- 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.

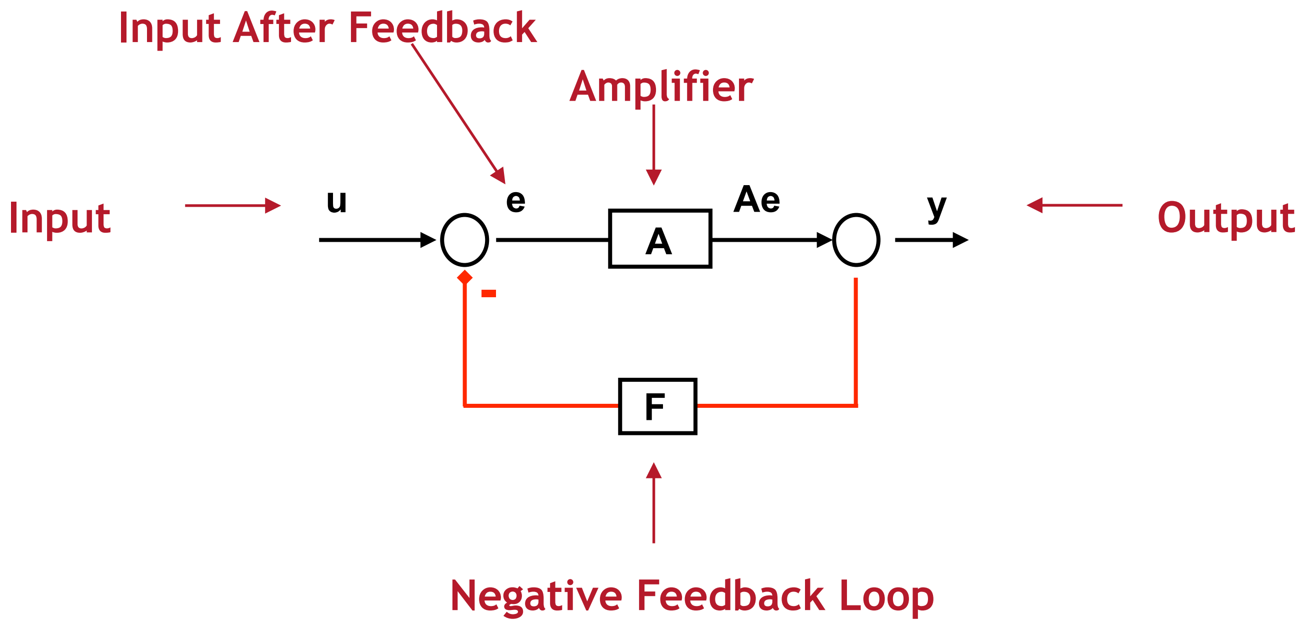
- The negative feedback loop results in a system that is much more robust to disturbances in the amplifier.

- 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

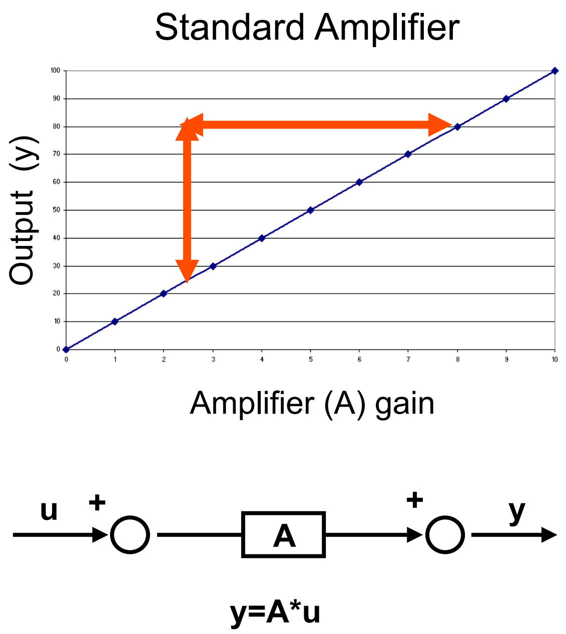


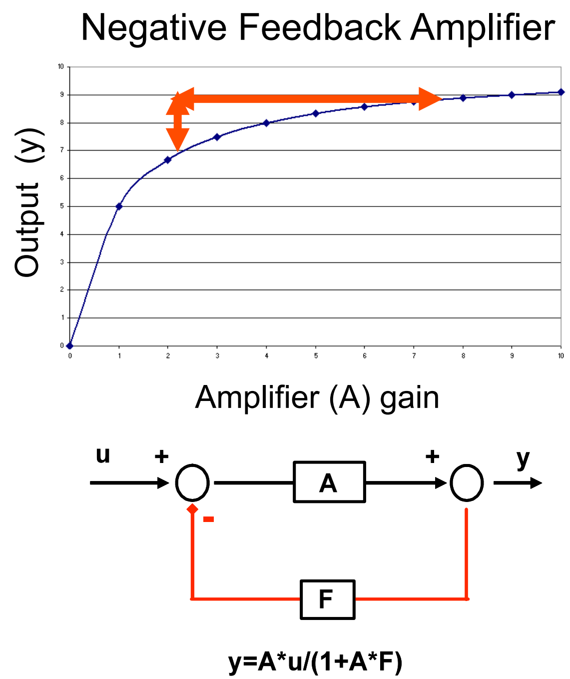
y = Ae
e = u - Fy

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

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

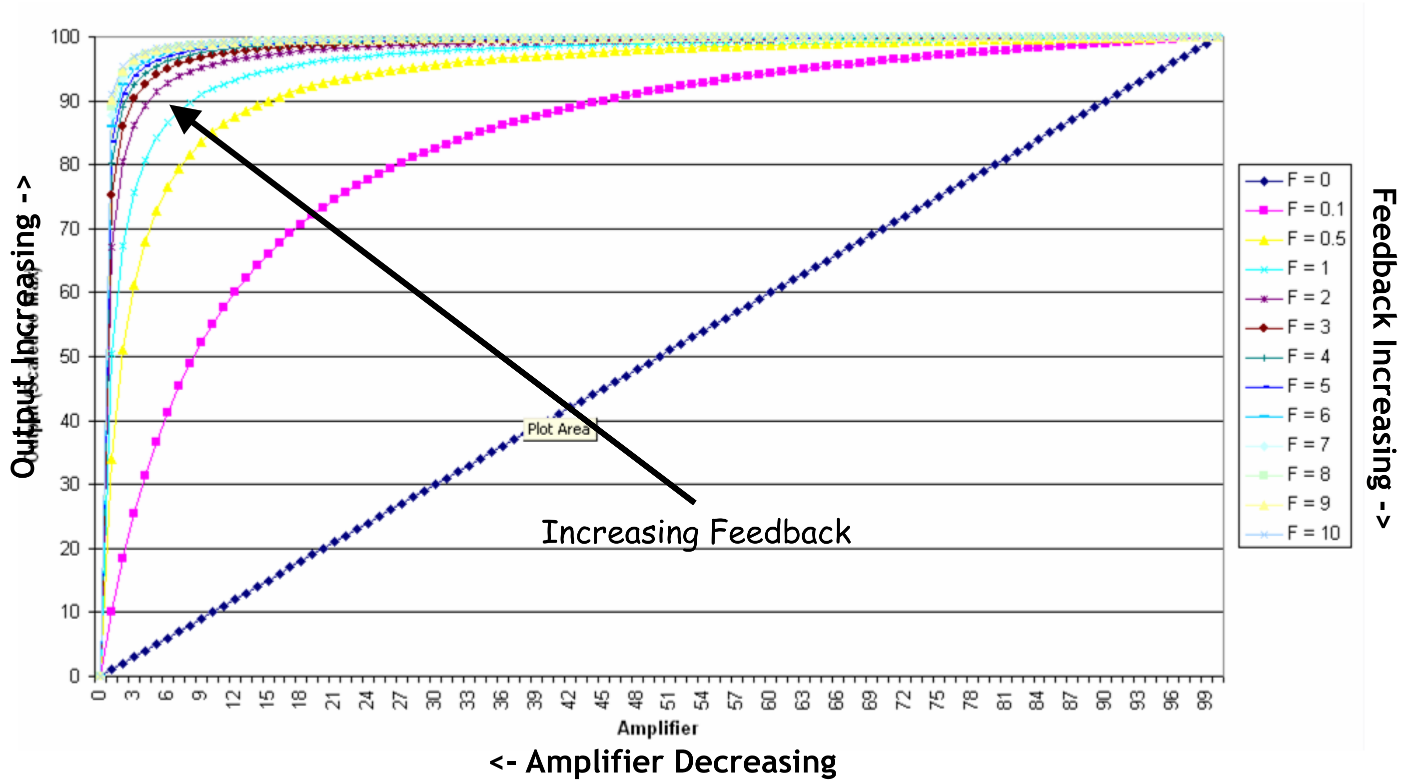
The negative feedback imparts signalling robustness





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

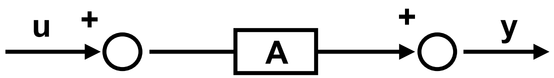
Feedback



Output Increasing ->
100
90
80
70
60
50
40
30
20
10
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78 81 84 87 90 93 96 99
Amplifier
<- Amplifier Decreasing
Plot Area
Increasing Feedback
F = 0
F = 0.1
F = 0.5
F = 1
F = 2
F = 3
F = 4
F = 5
F = 6
F = 7
F = 8
F = 9
F = 10
Feedback Increasing ->

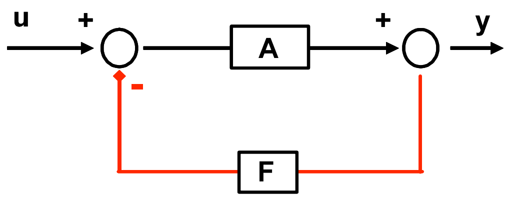
The negative feedback imparts signalling robustness

Standard Amplifier

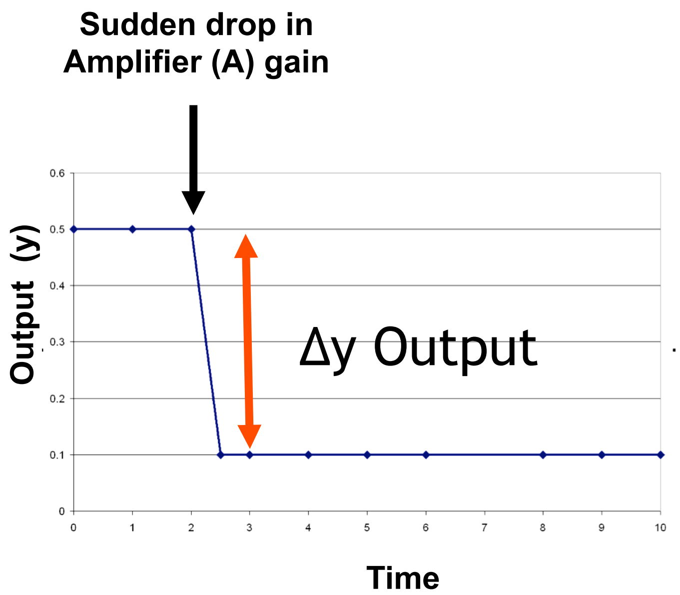


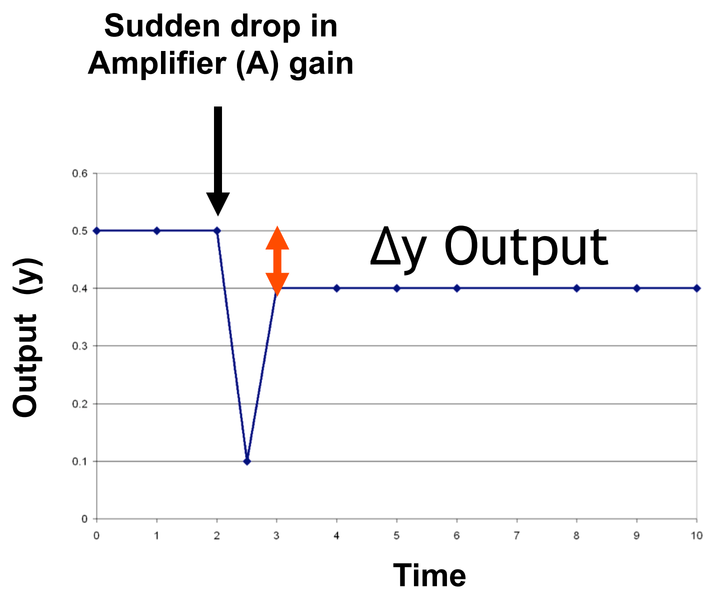
y=A*u

Negative Feedback Amplifier



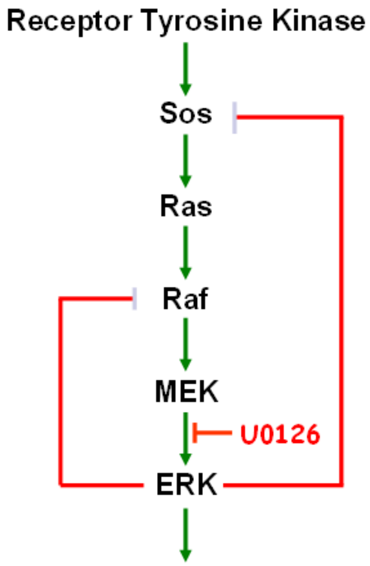
y=A*u/(1+A*F)



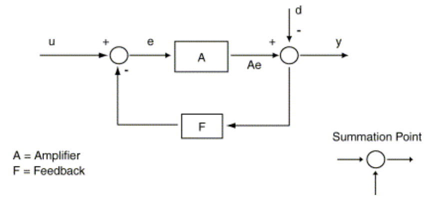


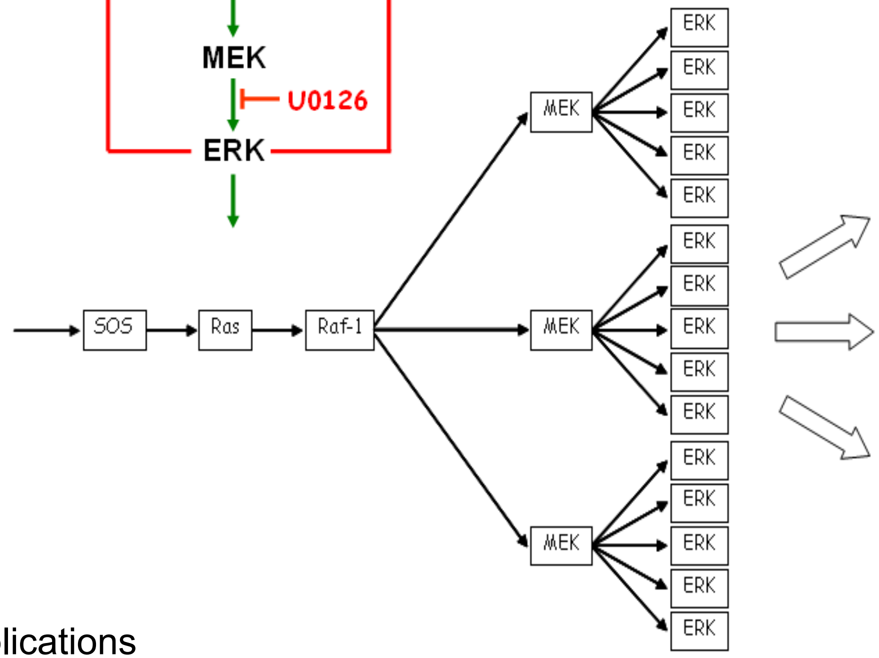
Application to Biology

- 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.

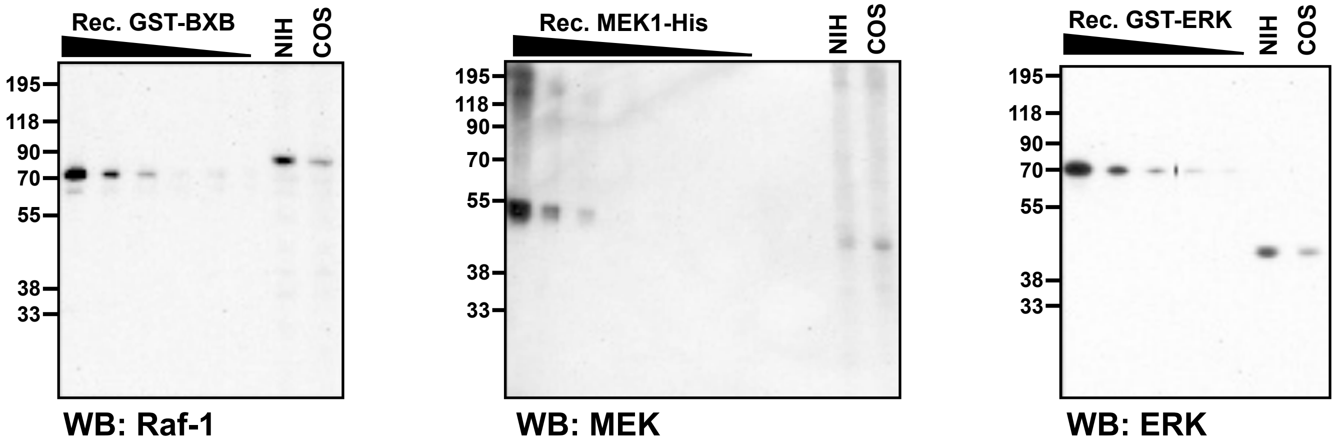


Sauro & Kholodenko (2004)





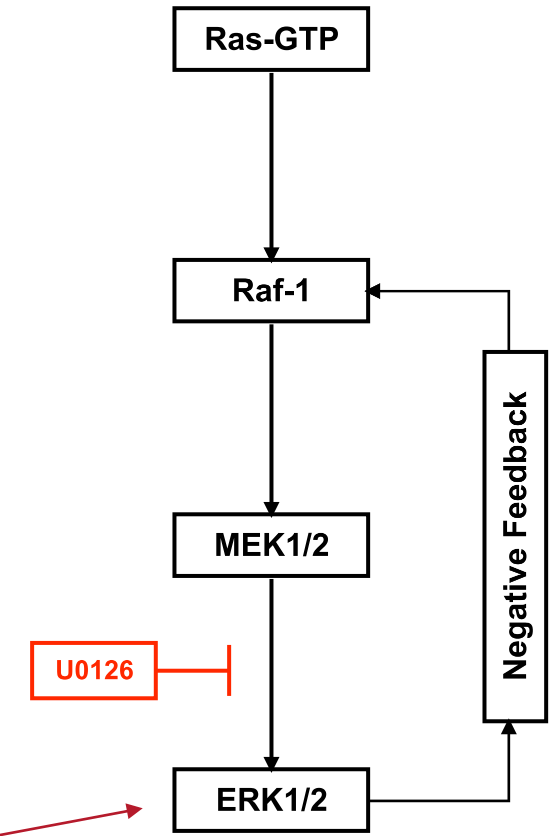
Raf/MEK/ERK amplifies the signal



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 to test if the ERK pathway is a NFA?

Generate input:
Stimulate with GF



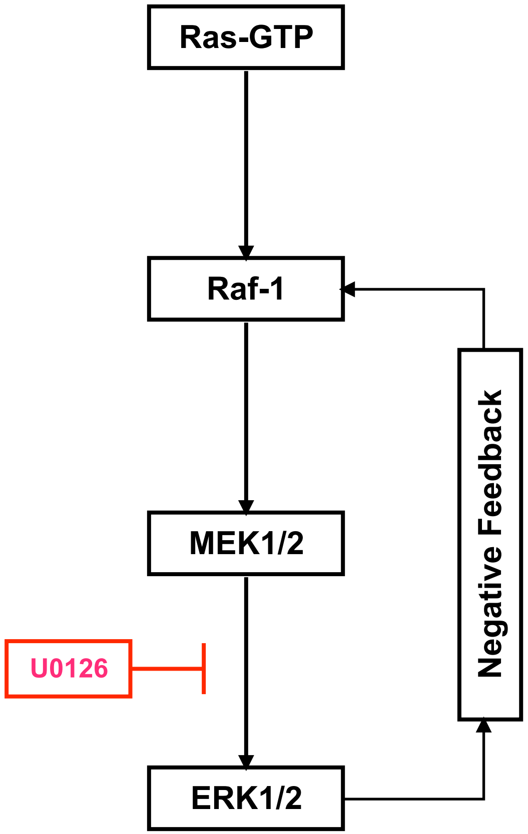
Remove negative feedback

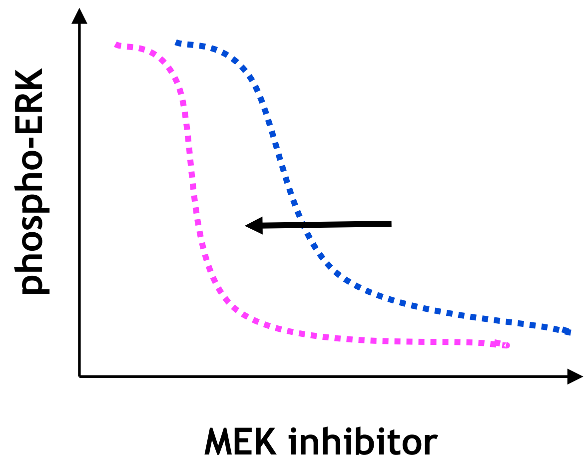
"Disturb the Amplifier": Use a MEK inhibitior, such as U0126

Measure signal output:
i.e. ERK phosphorylation

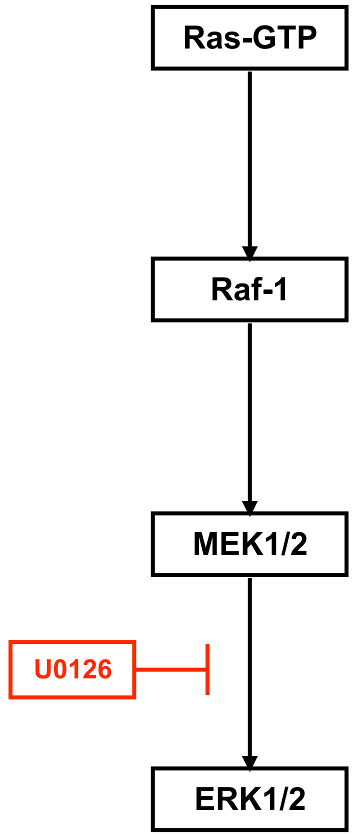
Hypothesis: Braking the feedback should sensitise the ERK pathway to MEK-inhibitor

Feedback intact





Feedback removed



How to test if the ERK pathway is a NFA?

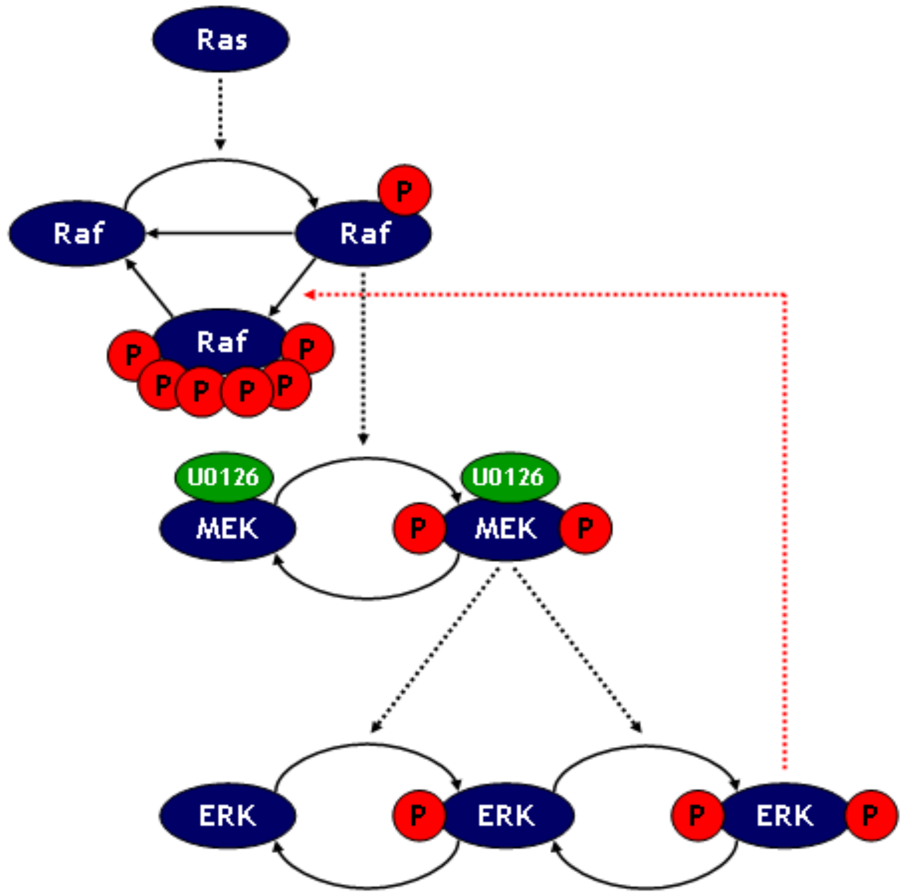
Strategy

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"

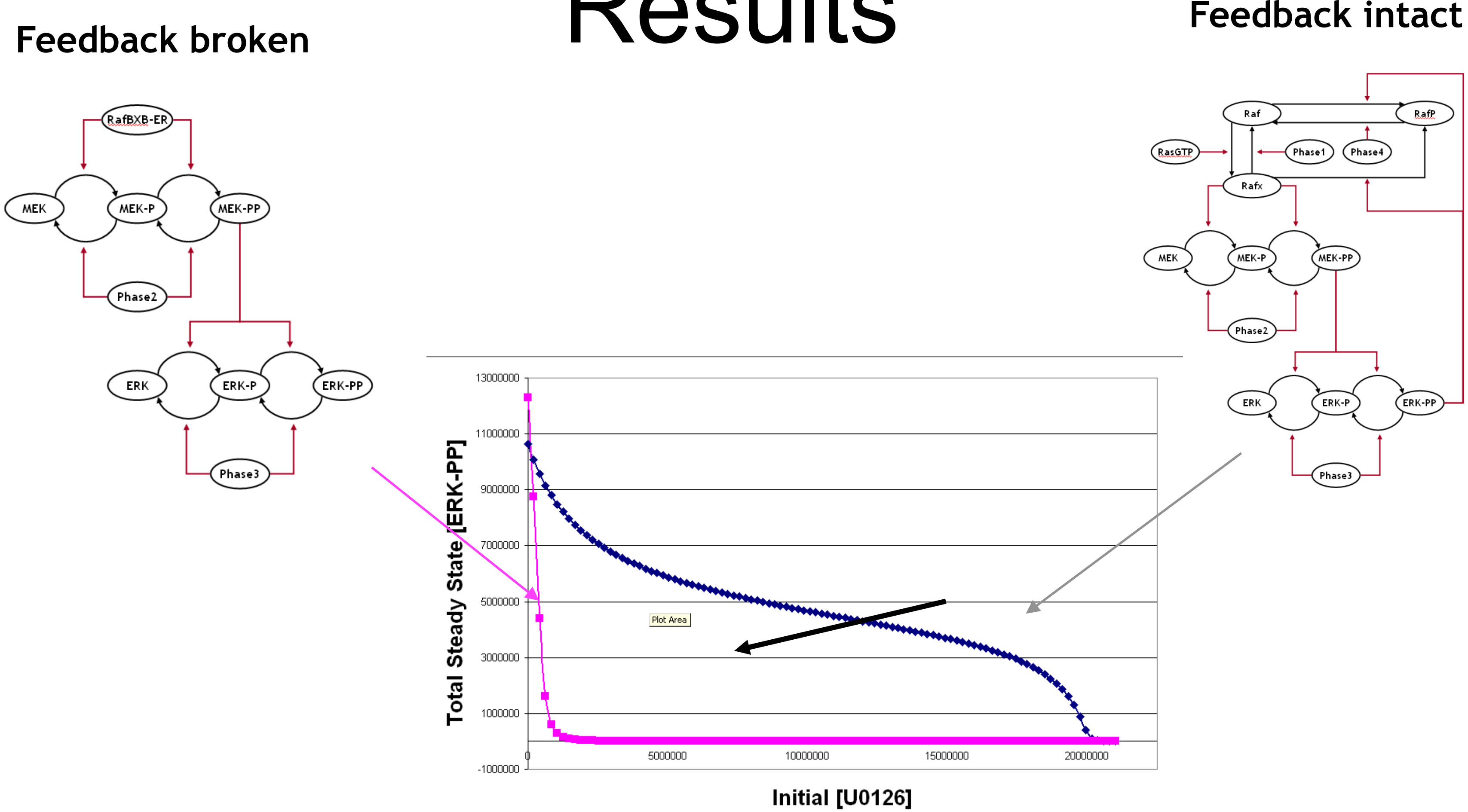


Schoeberl *et al*. (2002), Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors, Nature Biotechnology 20, 370-375

Amplifier / negative feedback

- Model amplifier strength by
  - Adding inhibitor to 2nd stage
  - Modifying kk3, kkk3 [I.e. modifying rate of production of RRp, RRRp]
  - Add/remove cascade elements
- Then plot amp strength versus output, e.g. [U] vs [RRRp]
- ?Model feedback strength by
  - Leaving out feedback loop
  - varying ki, and plot ki vs [RRRp]
- Notes: avoid saturation; use signal in linear range; ? model degradation in S1 signal?

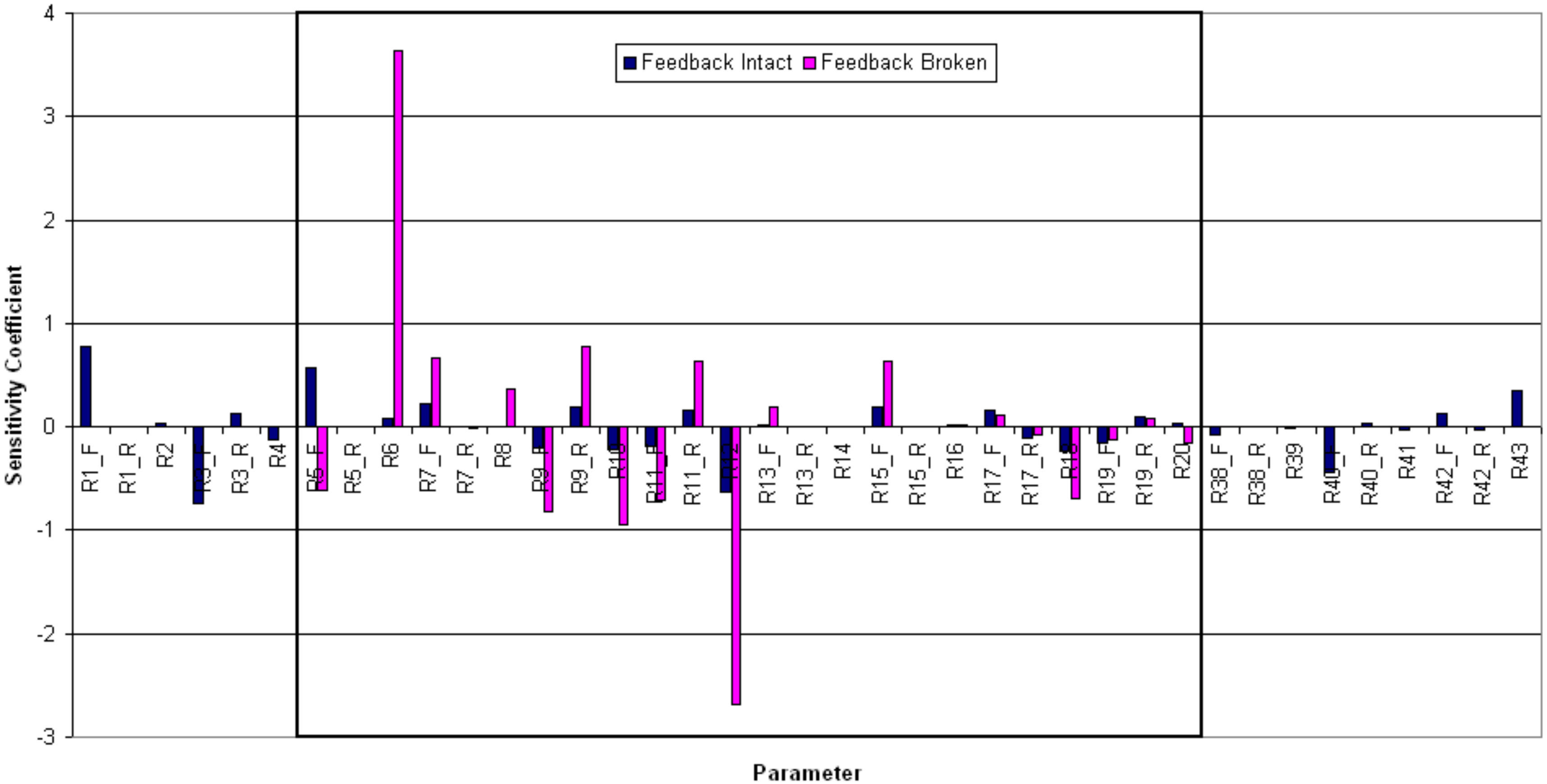
Computational Modeling 2: Results



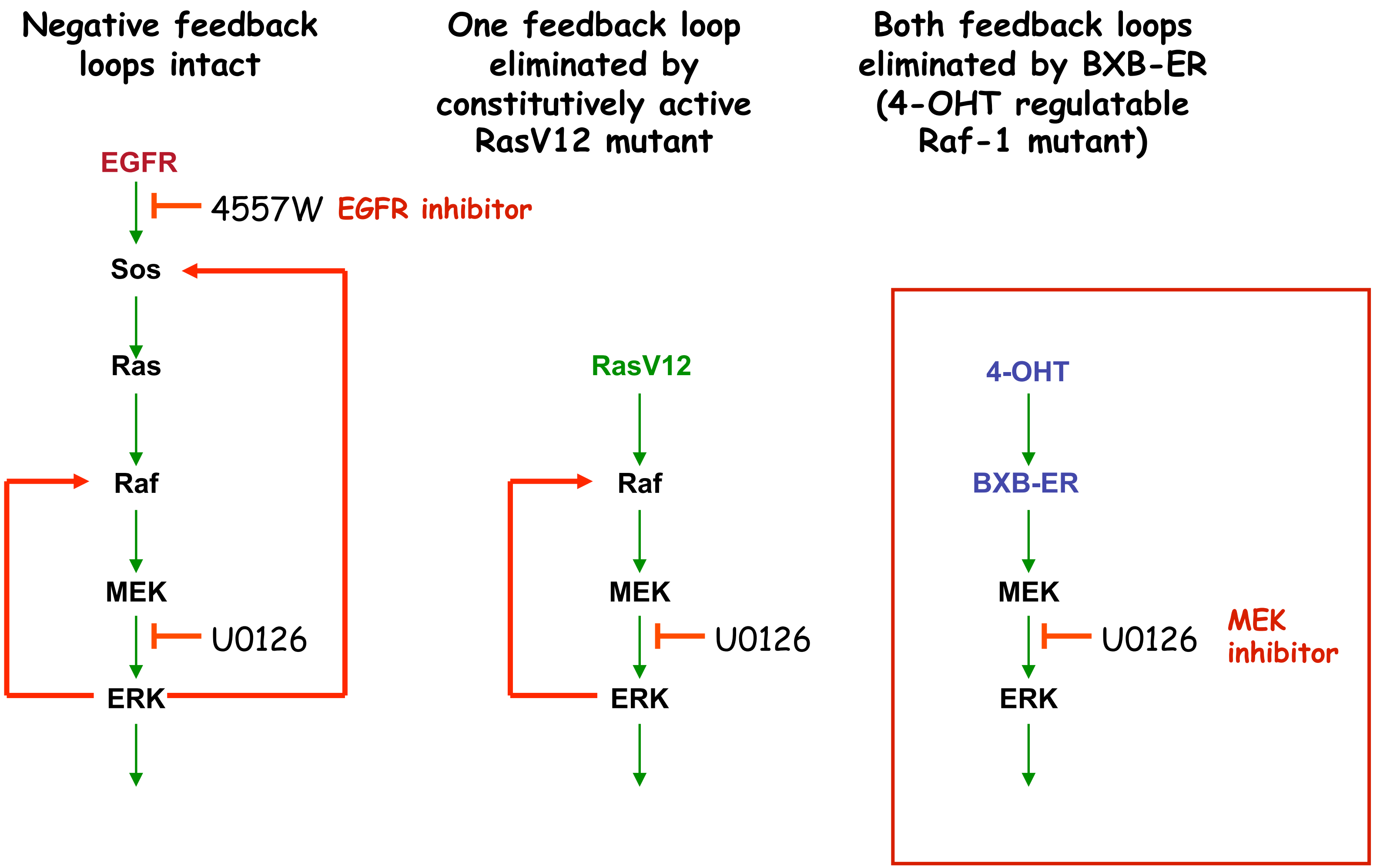
Prediction: Braking the feedback modulates drug response

Computational Modeling 2: Results

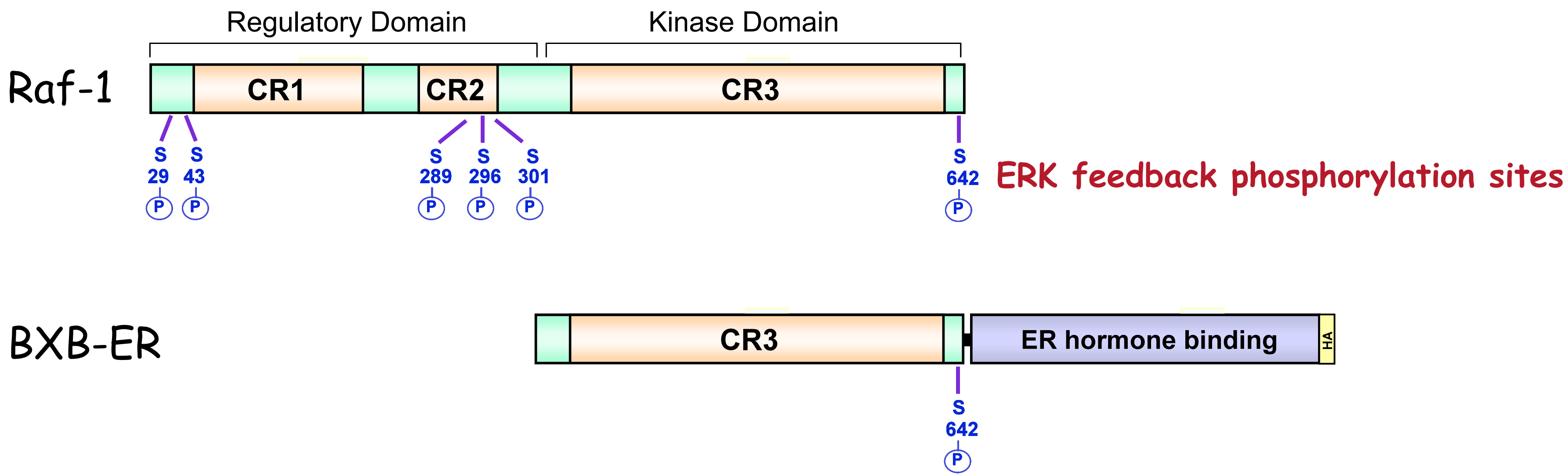
Sensitivity of kinetic parameters is decreased due to Negative Feedback

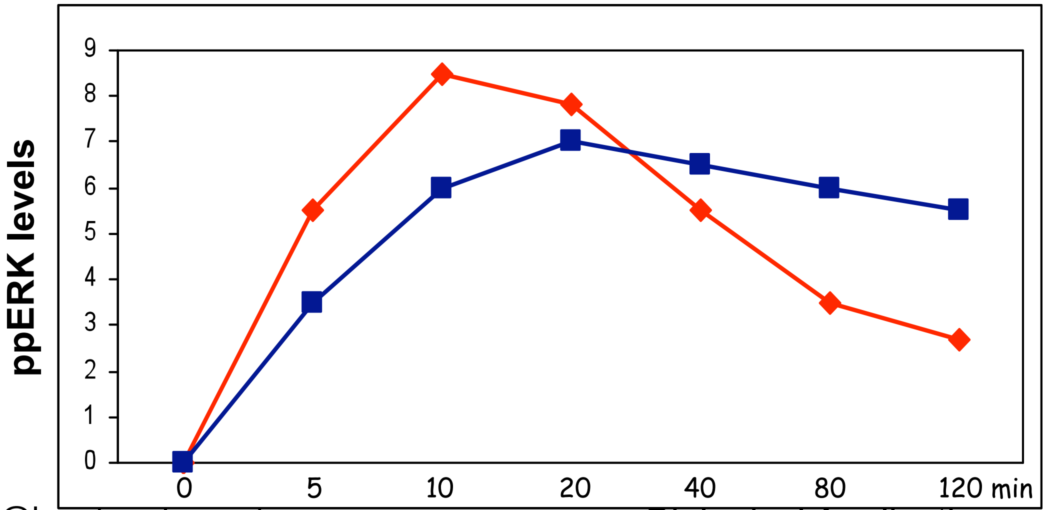


The experimental systems



Breaking the ERK feedback with BXBER

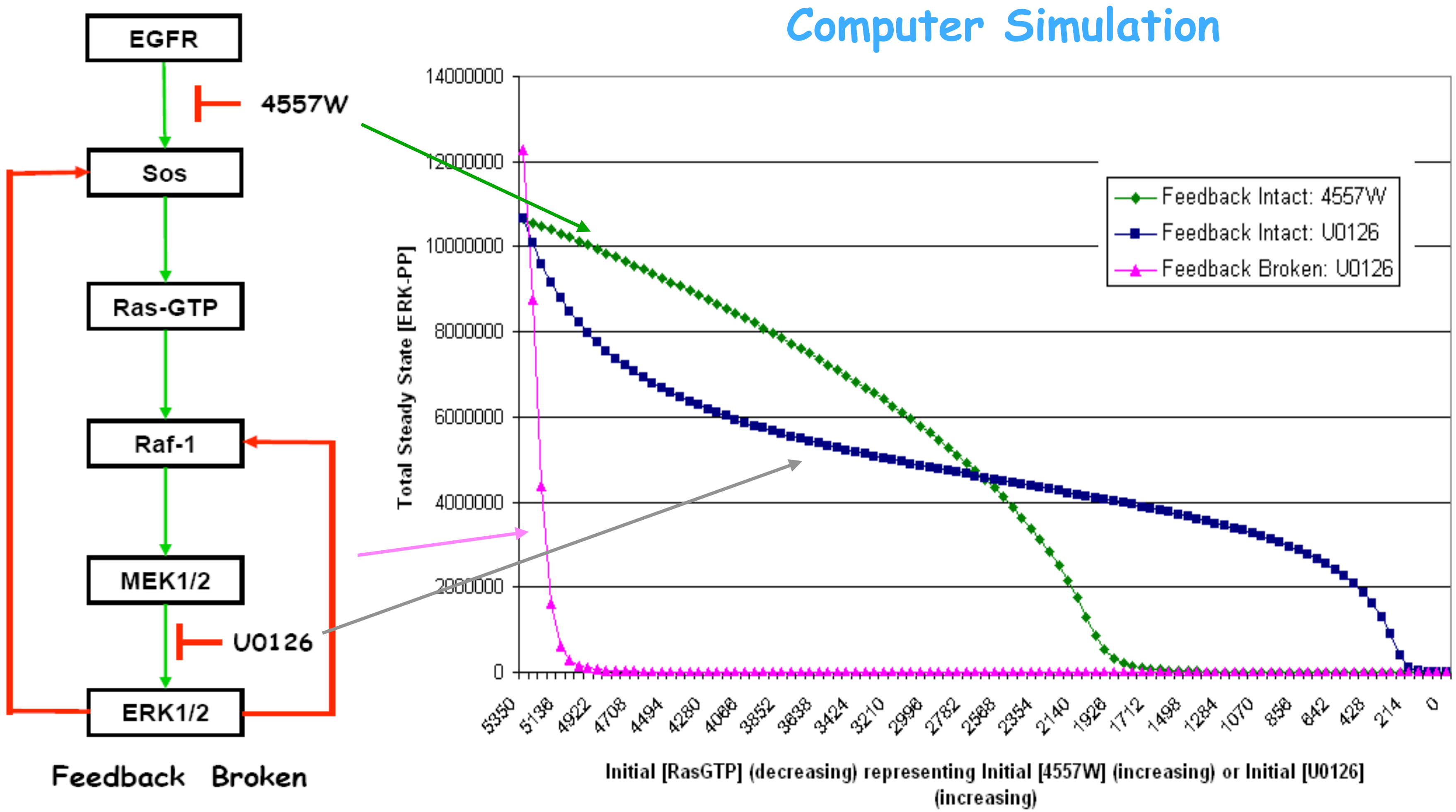




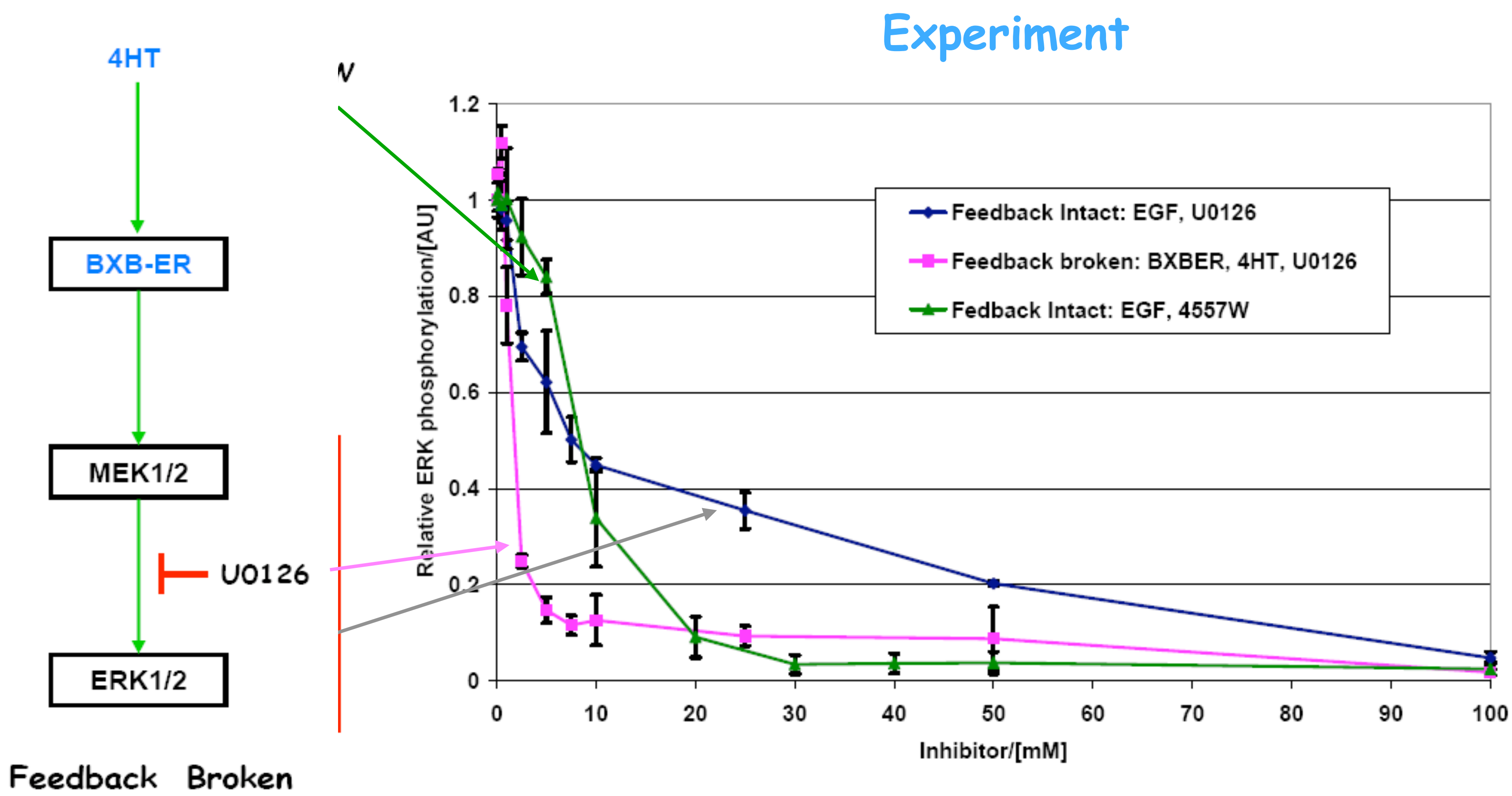
BXB-ER stimulated with 4-OHT
(4-Hydroxy Tamoxifen, a synthetic estrogen)

Raf-1 stimulated with EGF

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

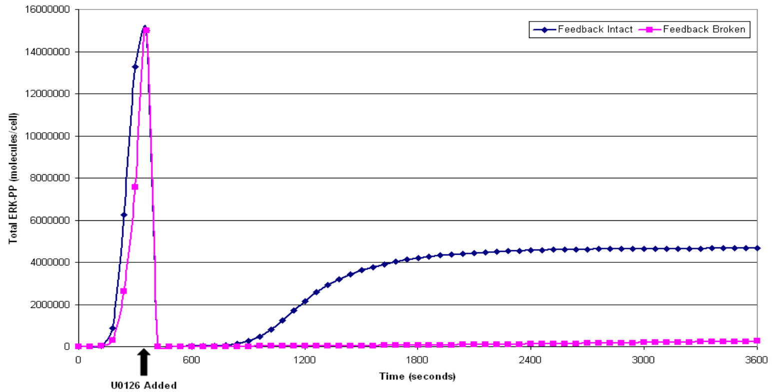


Ablation of feedback by BXBER decreases robustness to MEK-inhibitor U0126

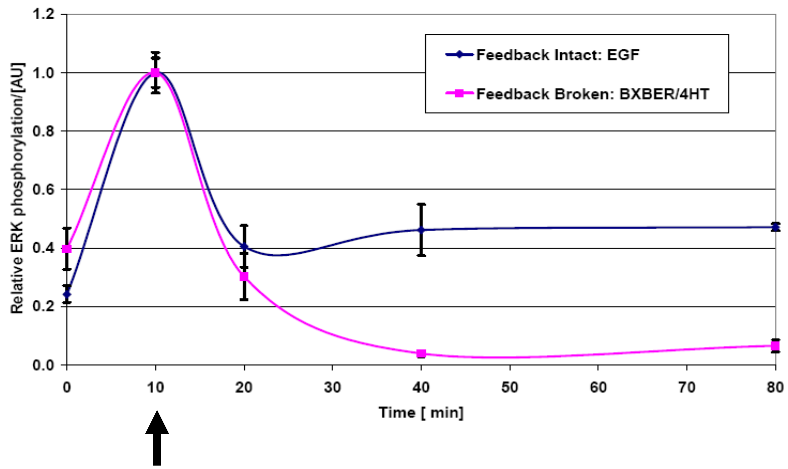


Signal recovery after MEK inhibition

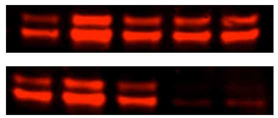
Simulation



Experiment



U0126 added

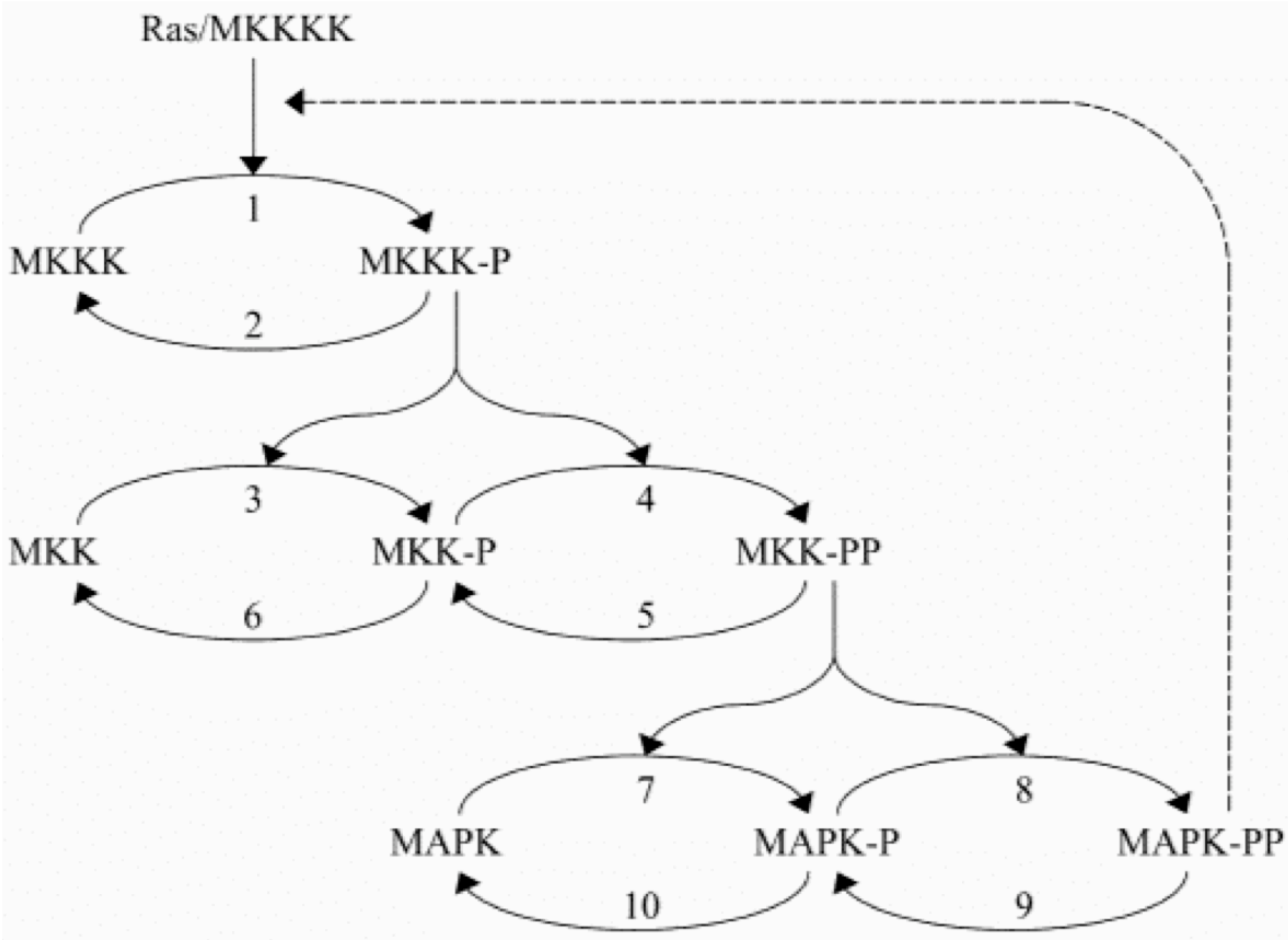


pERK1/2, +EGF

pERK1/2, + BXBER/4HT

0 10 20 40 80 min stimulation

Kholodenko – negative feedback oscillator



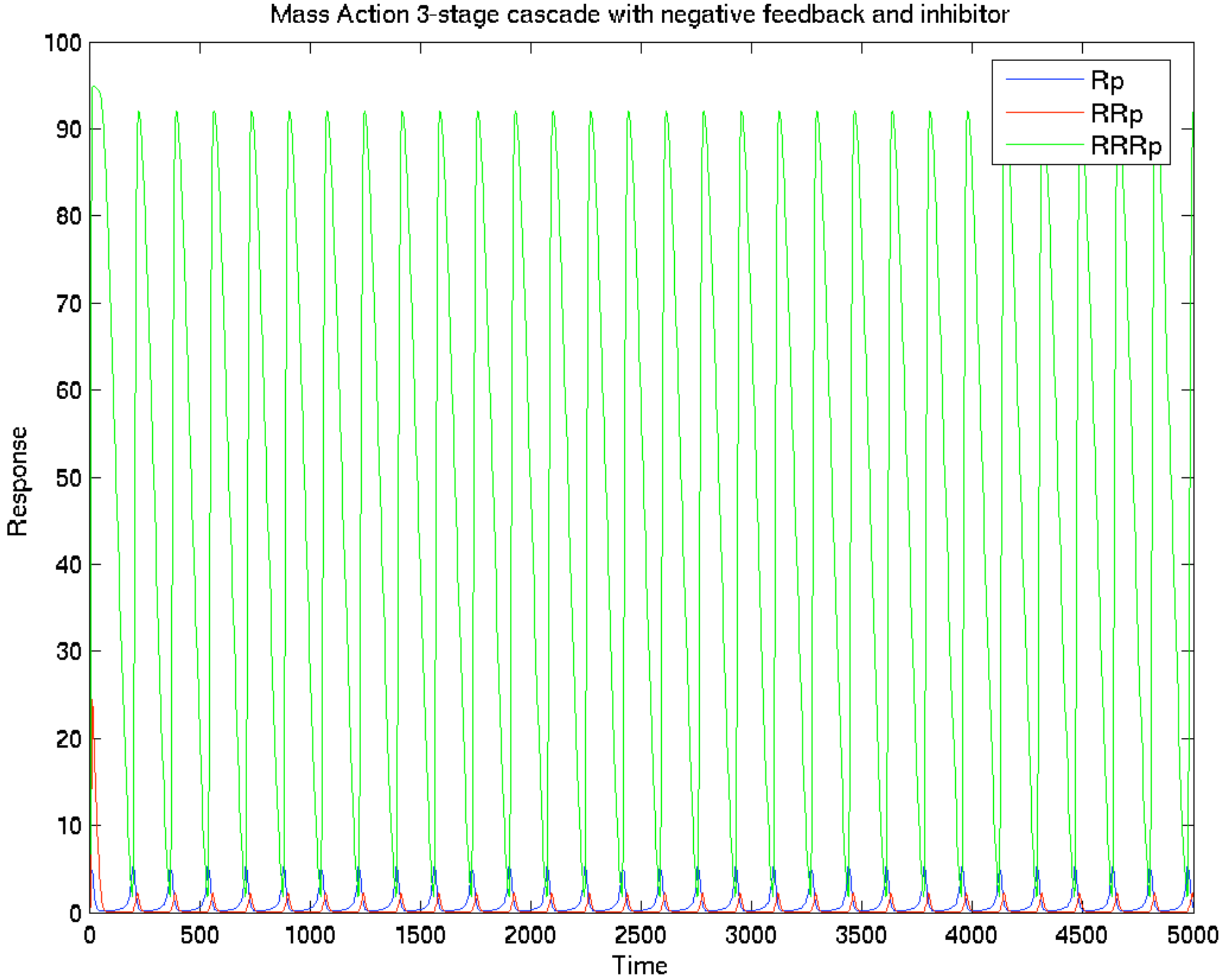
Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades.
Kholodenko BN., Eur J Biochem 2000 Mar;267(6):1583-8

Oscillations! Phosphorylation cascade + negative feedback: 3-stage, Inhibitor on 2nd stage, Mass Action

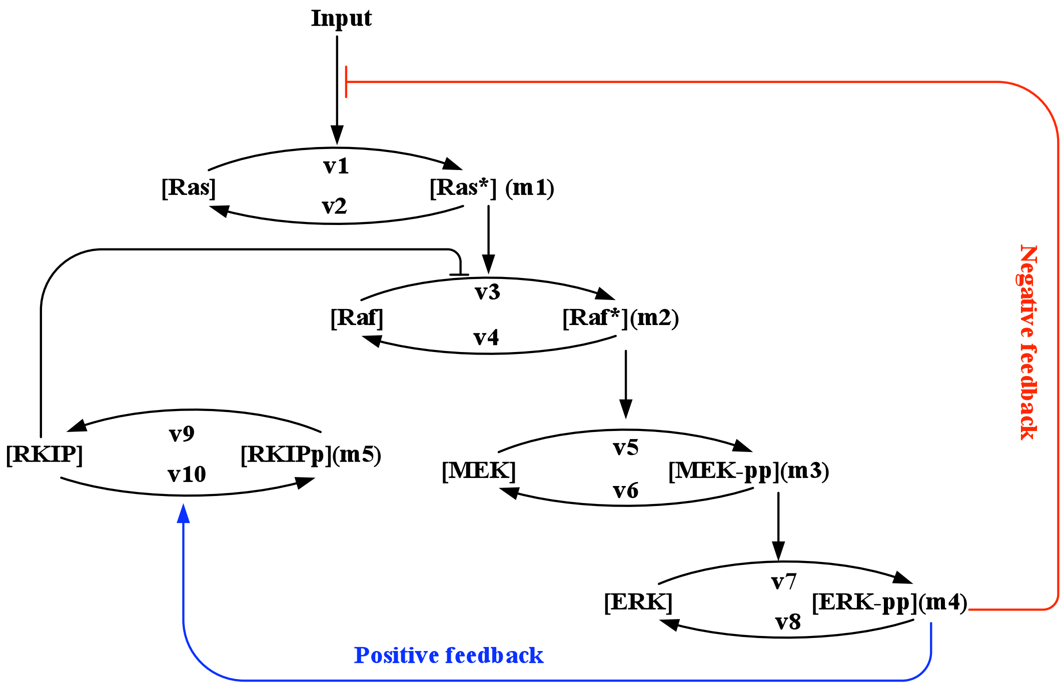
<u>Conditions</u>

S1=3

Inhibitor=0.5

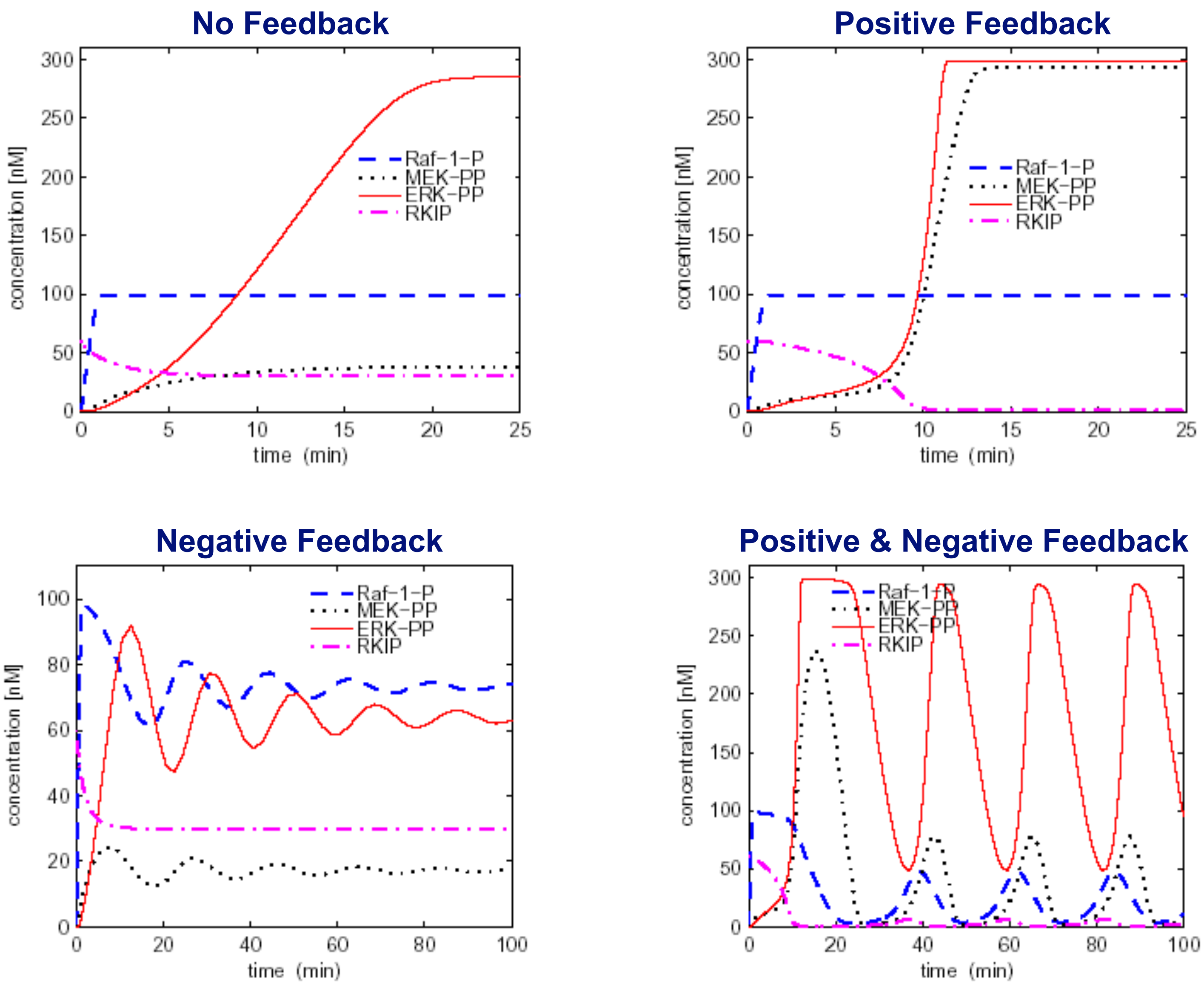


Combination of positive & negative feedback
Mathematical Model

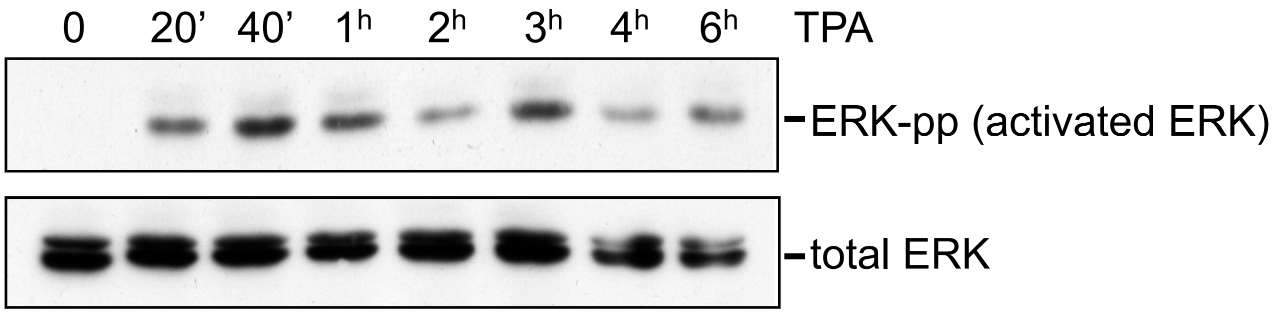


Modeling and Analysis of Two Feedback Loop Dynamics in Ras/Raf-1/MEK/ERK Signaling Pathway
Kwang-Hyun Cho, Sung-Young Shin, Walter Kolch, Olaf Wolkenhauer. ICSB 2004

Combination of positive & negative feedback: Simulation



Combination of positive & negative feedback: Simulation vs. Experimental Data



Western blots COS1 cell lysates