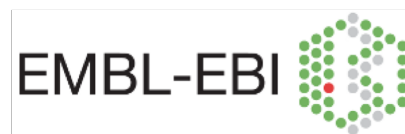# Building and Simulating Models using COPASI

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WTAC: In Silico Systems Biology Course, EMBL-EBI
9th – 14th July 2017

# Theme for this Tutorial: MAPK (Mitogen activated protein kinase) cascades

Mitogen activated protein kinase (MAPK) cascades are ubiquitous and highly conserved signalling modules, found in almost all eukaryotes. MAPK cascades are involved in many cellular processes such as cell proliferation, differentiation, movement, osmoregulation, survival etc.

In one well characterised signal transduction pathway, MAPK cascade couples the receptor (receptor tyrosine kinases (RTKs)) mediated events on the cell surface to cytoplasmic and nuclear effectors (Figure 1).

In response to stimuli, phosphorylated RTKs complexed with GRB2, recruit the cytoplasmic guanine nucleotide exchange protein Son of Sevenless (SOS) to the cell membrane, which then activates the membrane bound GTPase RAS.

Activated RAS triggers the activation of a MAPKKK (Raf) and starts the MAPK cascade. The signal-relay mechanism involves sequential phosphorylation of three kinases. Both the MAPKK and the MAPK have to be phosphorylated on at least two sites (a conserved tyrosine and a threonine residue) to be active.

The cascade arrangement has important consequences for the dynamics (switch like or all-or-none and oscillatory activation pattern) of MAPK signalling.
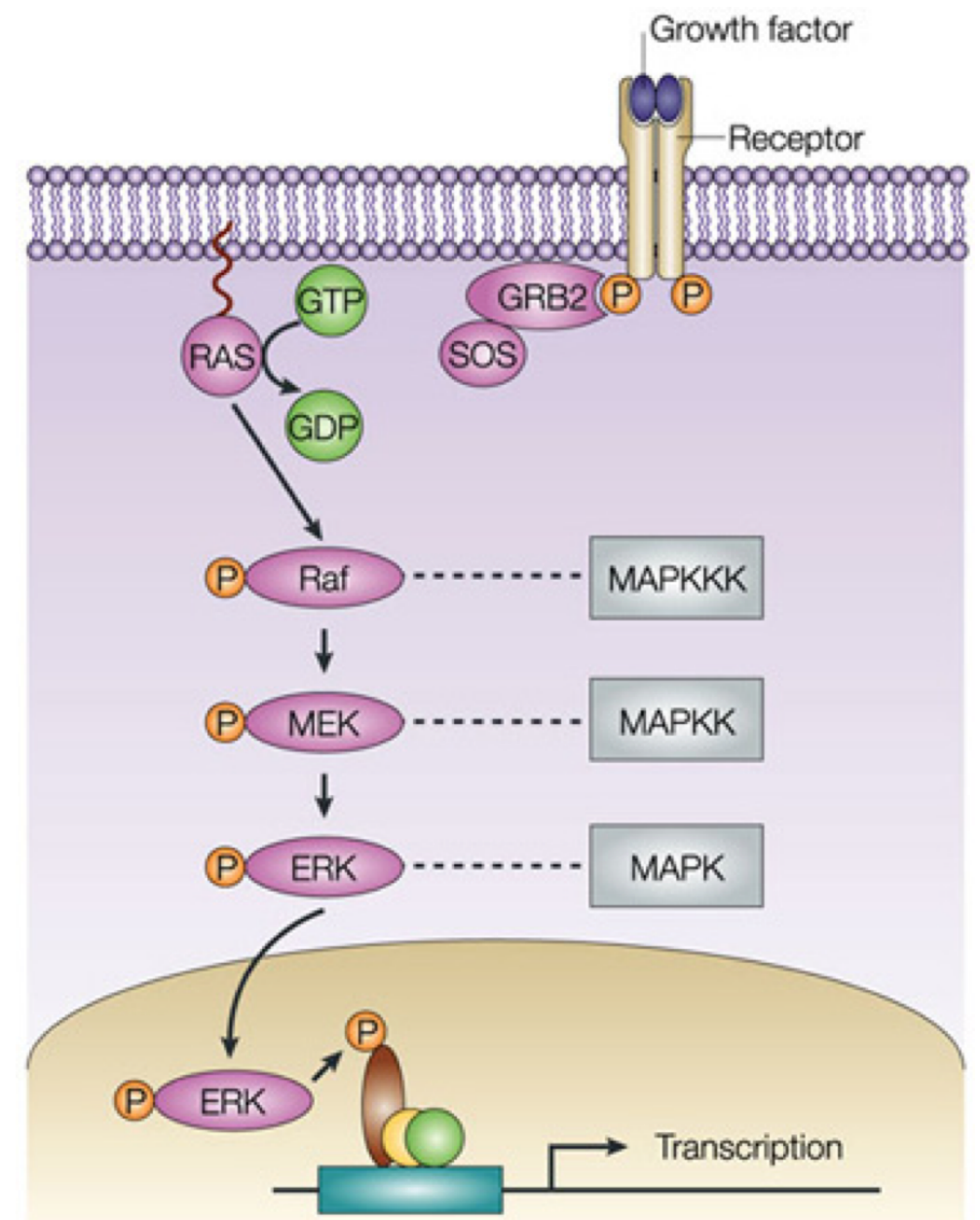
Nature Reviews | Molecular Cell Biology

Figure 1: MAPK cascade. Figure from http://www.nature.com/nrm/journal/v5/n6/box/nrm1400_BX1.html

Tools used in this Tutorial: COPASI

COPASI (stands for "Complex Pathway Simulator"). It is an open-source software widely used in computational systems biology because of its versatility and ease of use.

It comes with a command-line version that can be used for instance on clusters, and a user friendly graphical interface - which we will use today.

The tool is used for teaching but also for research (the main paper, Hoops et al 2006 has been cited > 1400 times). Plenty of information including manuals, videos and user forums is available at:

http://www.copasi.org

Latest version: 4.19 (Build 140)

Models Used In This Tutorial

BioModels Database

Some of the example models used for this tutorial are taken from the BioModels database (https://www.ebi.ac.uk/biomodels). BioModels contains thousands of mathematical models covering a large diversity of biological processes. Many of them have been carefully verified and annotated, and are a great starting point for further modelling endeavours.

In particular, we will use:

BIOMD0000000009 – Huang 1996
Huang CY, Ferrell JE Jr. Ultrasensitivity in the mitogen-activated protein kinase cascade. Proc Natl Acad Sci U S A. 1996 Sep 17;93(19):10078-83. (http://www.ebi.ac.uk/biomodels-main/BIOMD0000000009)

BIOMD0000000010 - Kholodenko2000
Kholodenko BN. Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. Eur J Biochem. 2000 Mar;267(6):1583-8. (http://www.ebi.ac.uk/biomodels-main/BIOMD0000000010)

BIOMD0000000027 - Markevich2004
Markevich NI,Hoek JB, Kholodenko BN. Signalling switches and bistability arising from multisite phosphorylation in protein kinase cascades. J Cell Biol. 2004 Feb 2;164(3):353-9.
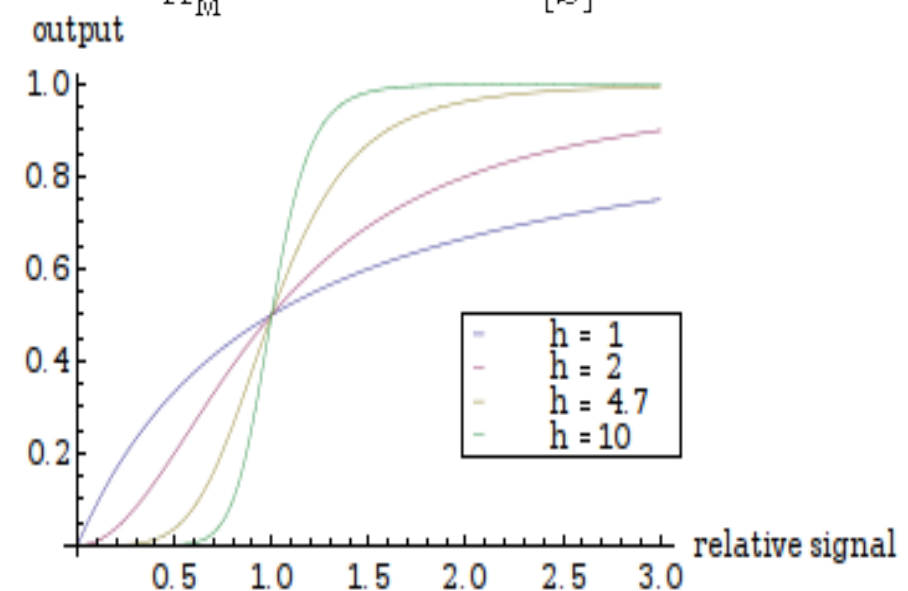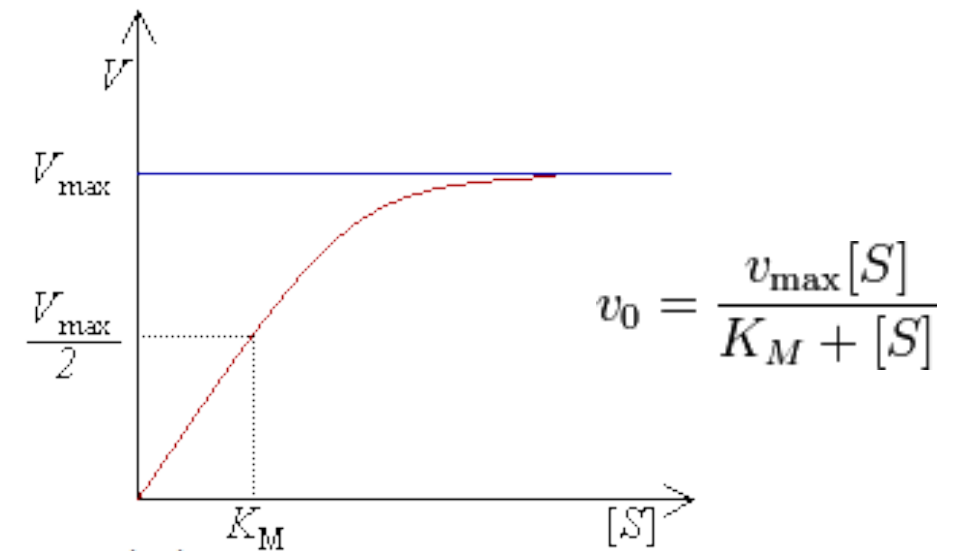(http://www.ebi.ac.uk/biomodels-main/BIOMD0000000027).

BIOMD0000000009 – Huang1996 - Ultrasensitivity in MAPK Cascades

This model discusses ultrasensitivity in MAPK cascades.

Ultrasensitivity is defined as a the steady state behaviour of a system where the output of a system remains low until the system crosses a threshold input value. Once the input crosses the threshold value, the output signal becomes extremely sensitive to small changes in stimulus strength, and results in a sigmoidal (S-shaped) input - output curve.

This is comparable to Hill's equation, where hill co-efficient h>1 represents ultrasensitivity.

There are several processes that result in ultrasensitivity. For example: cooperativity during binding of haemoglobin to its substrates and when enzyme cycles operate near saturation where the stimulus acts upon multiple steps of an enzyme cascade (eg. MAPK cascade).
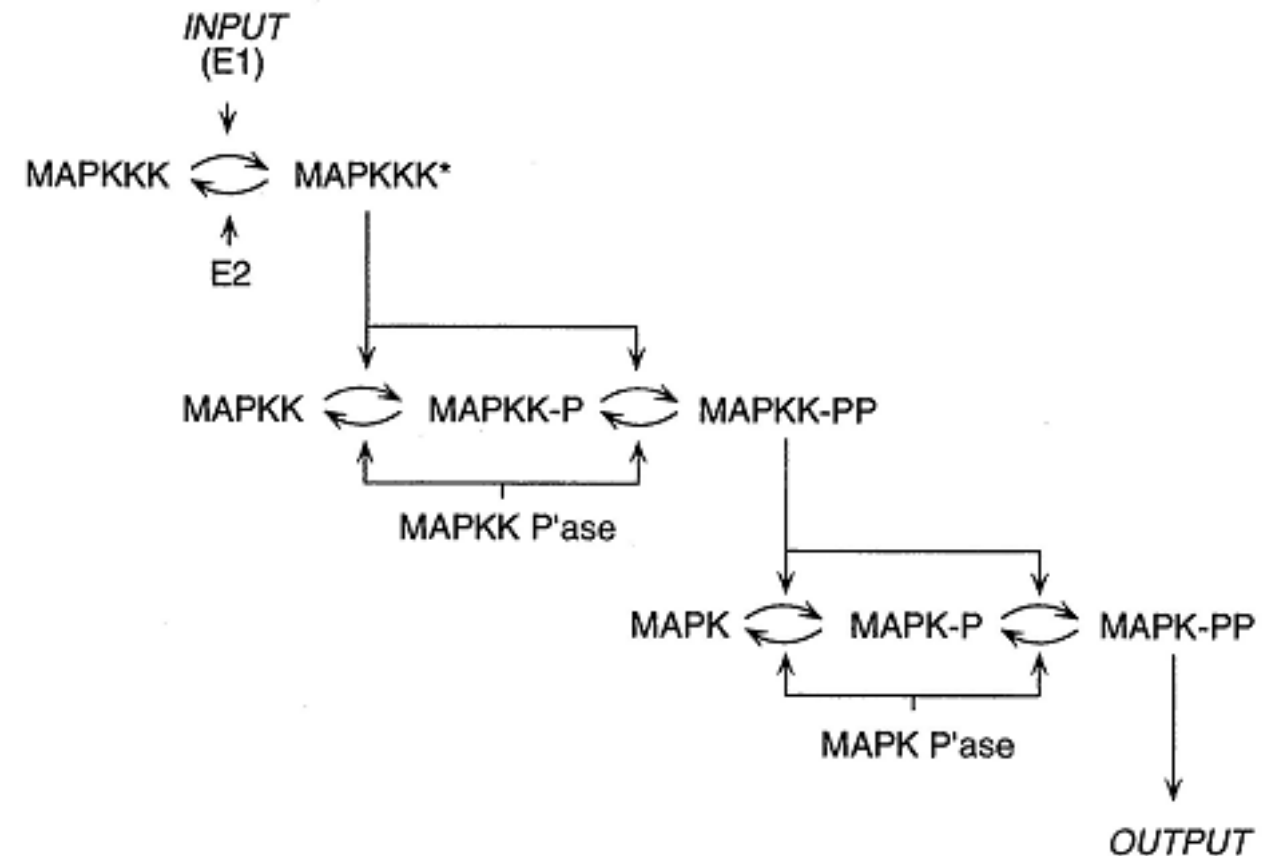
$$v_0 = \frac{v_{\max}[S]}{K_M + [S]}$$

$$out = s^h / \{1 + s^h\}$$

BIOMD0000000009 – Huang1996 - Ultrasensitivity in MAPK Cascades

The cascade is modelled as a simple linear chain of successive phosphorylations and de phosphorylations.

Mass action kinetics for kinase and phosphatase reactions.

The cascade arrangement has important consequence on the dynamics of the system.

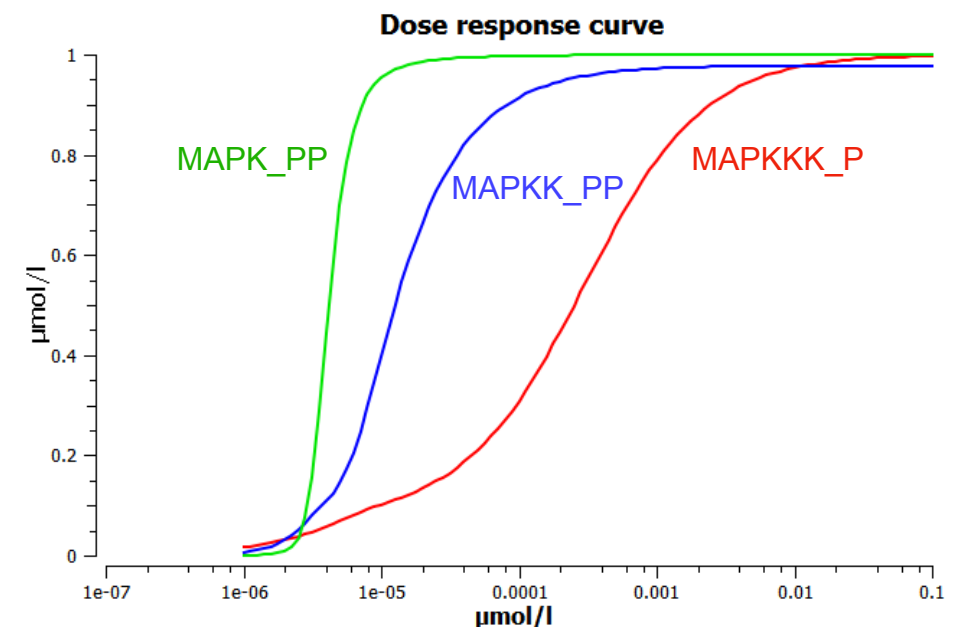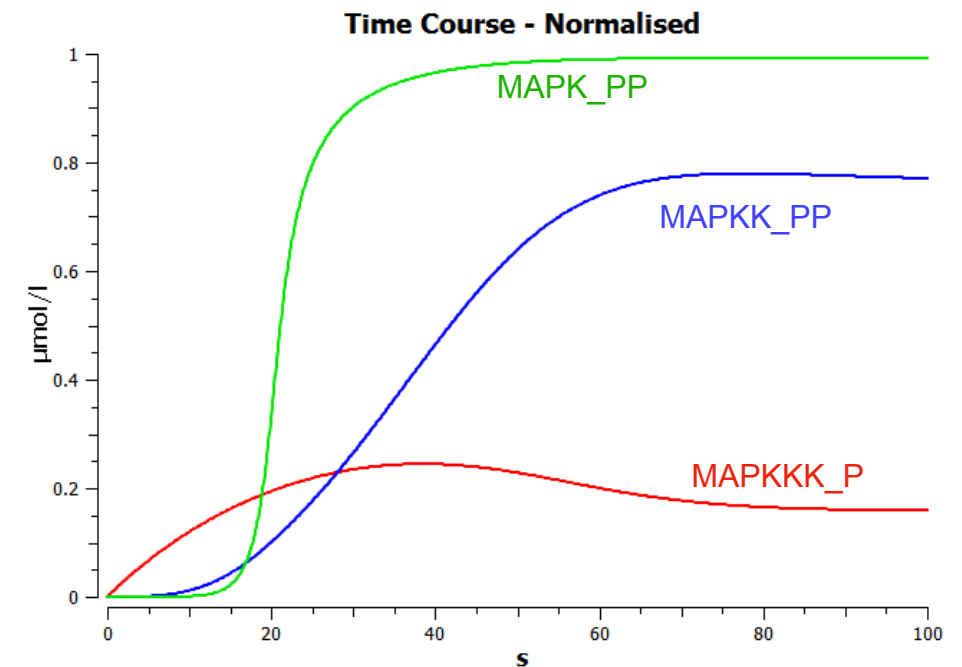# BIOMD0000000009 – Huang1996 - Ultrasensitivity in MAPK Cascades

What do we infer ?

The temporal sequence of kinase activation, is from MAPKKK to the final effector MAPK.

Increase in sensitivity along the levels of the cascade; MAPK reaches its maximal level before MAPKKK.

The dose-response curves (varying stimulus E1, i.e. Ras (MAPKKK_activator) predicted to be sigmoidal

MAPK_PP - Hill's coefficient nearly 5 (steepest).
MAPKK_PP– Hill's coefficient 1.7
MAPKKK _P– linear Michaelis-Menton enzyme (Hill's coefficient 1)

BIOMD0000000010 - Kholodenko2000 - Ultrasensitivity & Negative feedback

Feedback loops:

Positive feedback: increase or activation of a downstream element, causing the same effect (i.e. increase or activation) to an upstream element, in a sequence of biological process.

Negative feedback: increase or activation of a downstream element, causing an opposite effect (i.e. decrease or inactivation) to an upstream element, in a sequence of biological process.
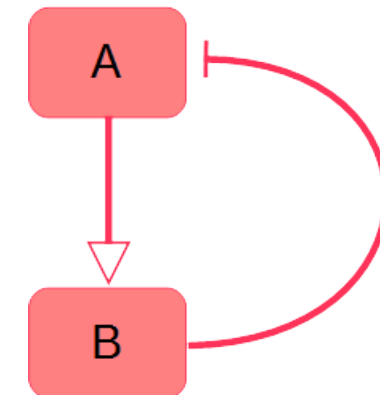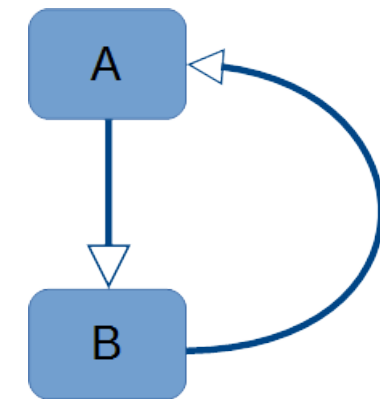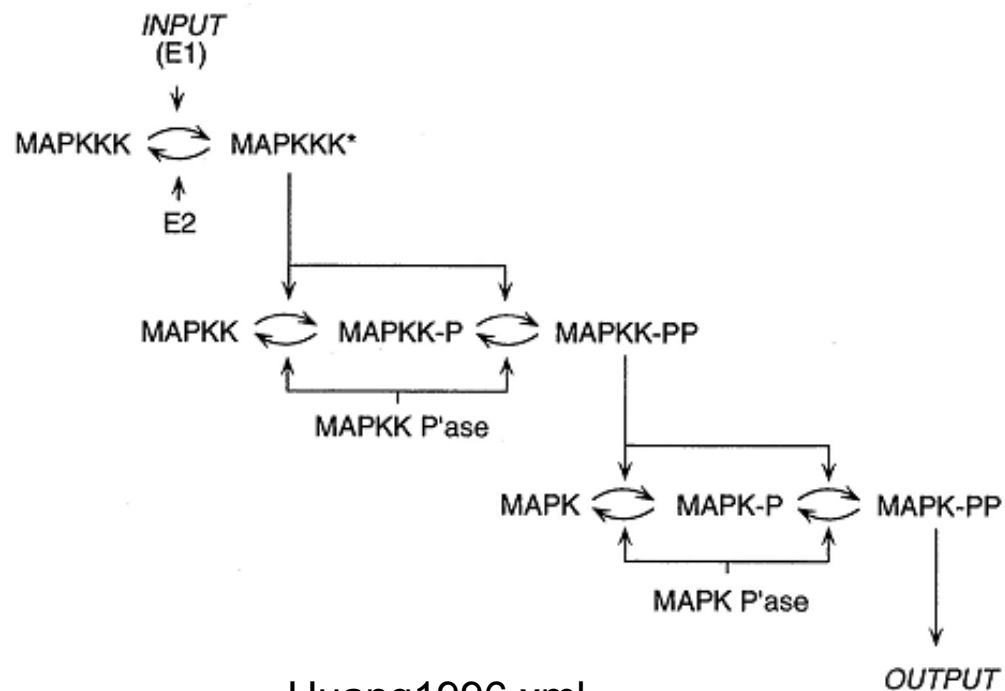
BIOMD0000000010 - Kholodenko2000 - Ultrasensitivity & Negative feedback

The cascade is modelled as a linear chain of successive phosphorylations and de phosphorylation.

Inhibitory phosphorylation of SOS by MAPK (ERK), switches off Ras signalling. Indeed, whereas activated Raf (MAPKKK) brings in ERK activation, ERK mediated inhibition of Raf stimulation by SOS decrease ERK phosphorylation.

Michaelis Menten kinetics for kinase and phosphatase reactions.

Huang1996.xml


Negative Feedback loop
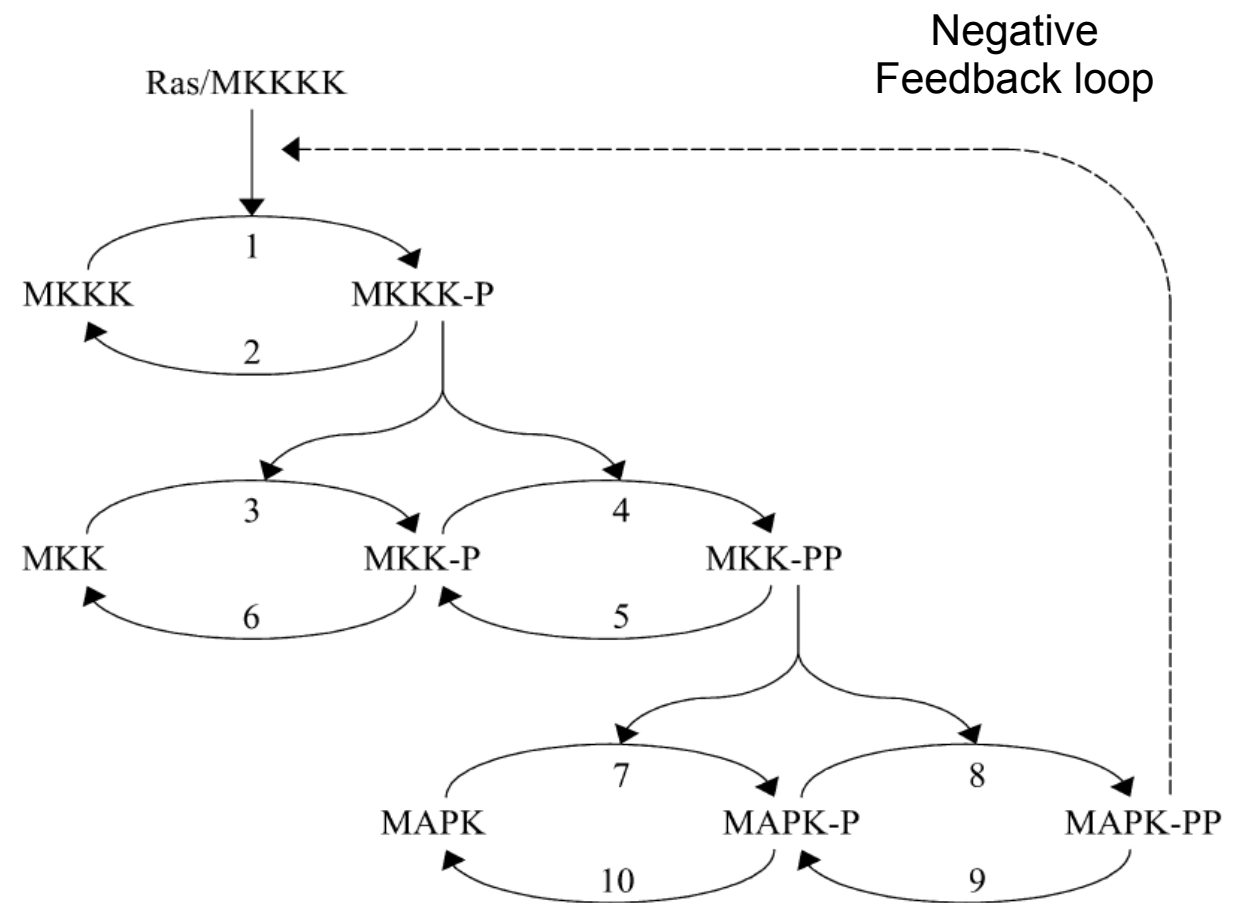
# BIOMD0000000010 - Kholodenko2000 - Ultrasensitivity & Negative feedback

With a simple negative feedback, the system exhibits oscillatory behaviour under constant stimulation. i.e. The combination of ultrasensitivity and negative feedback brings sustained biochemical oscillations.

With smaller inhibition constants (Ki), the coupling between ERK and Raf decreases and the frequency of oscillations increases.
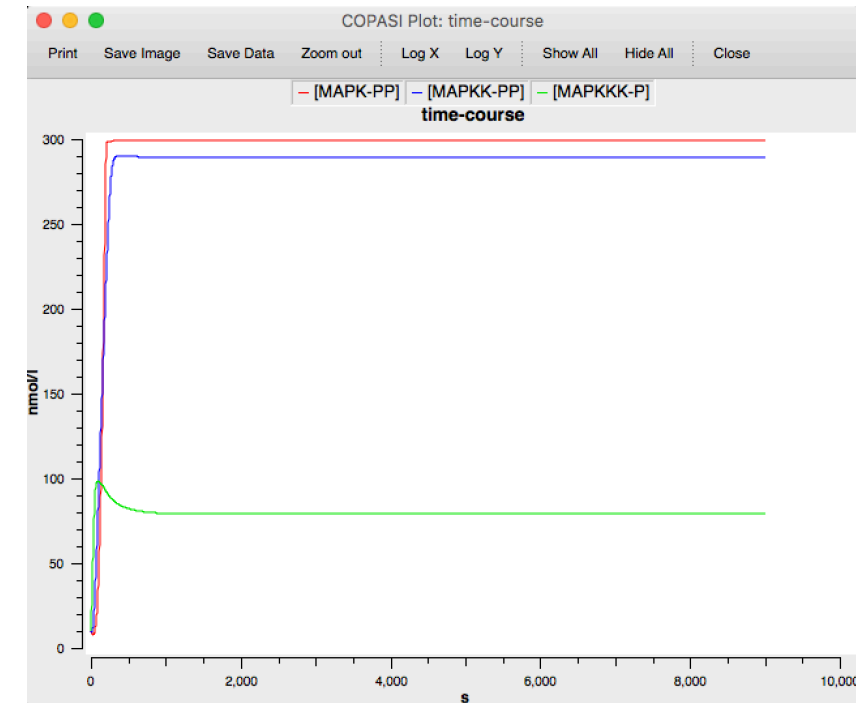
—— **MAPKK-PP**
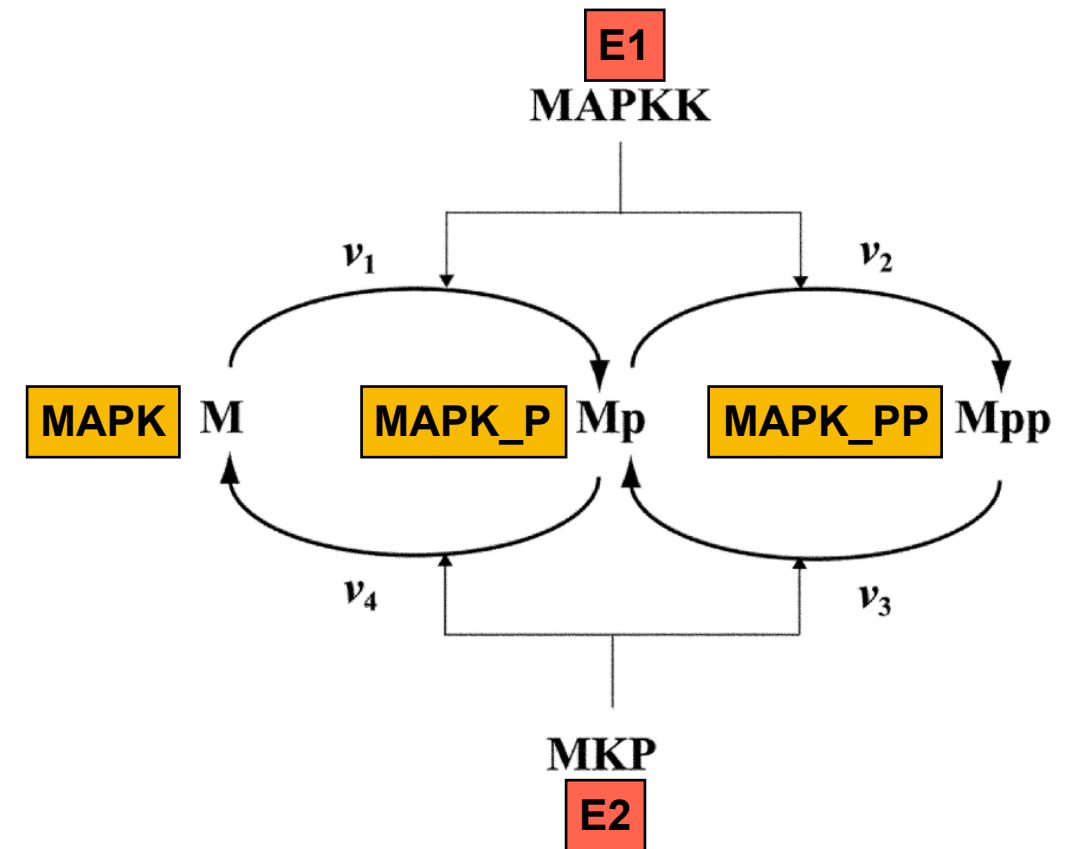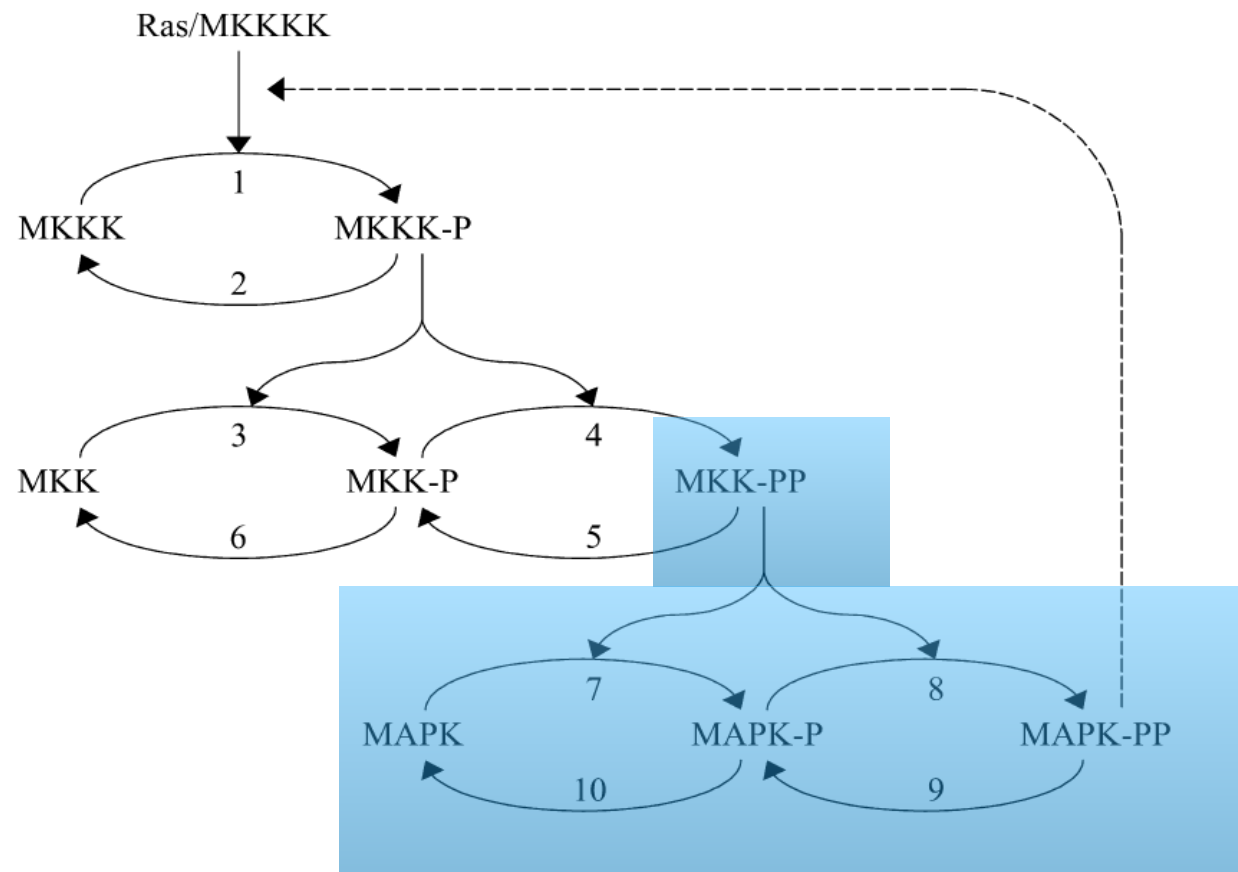
—— **MAPKKK-P**

—— **MAPK-PP**
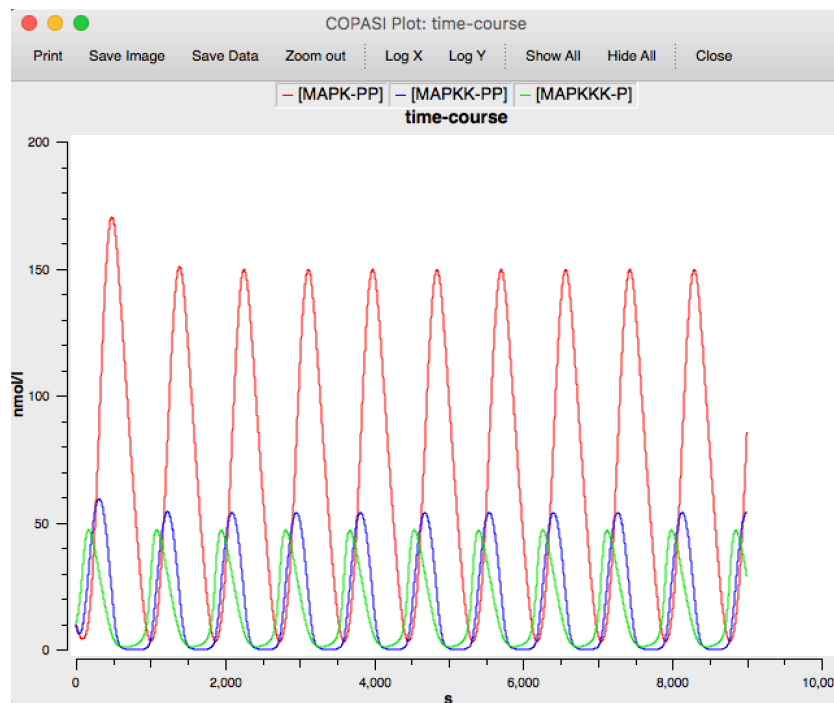
Ki = 1.324
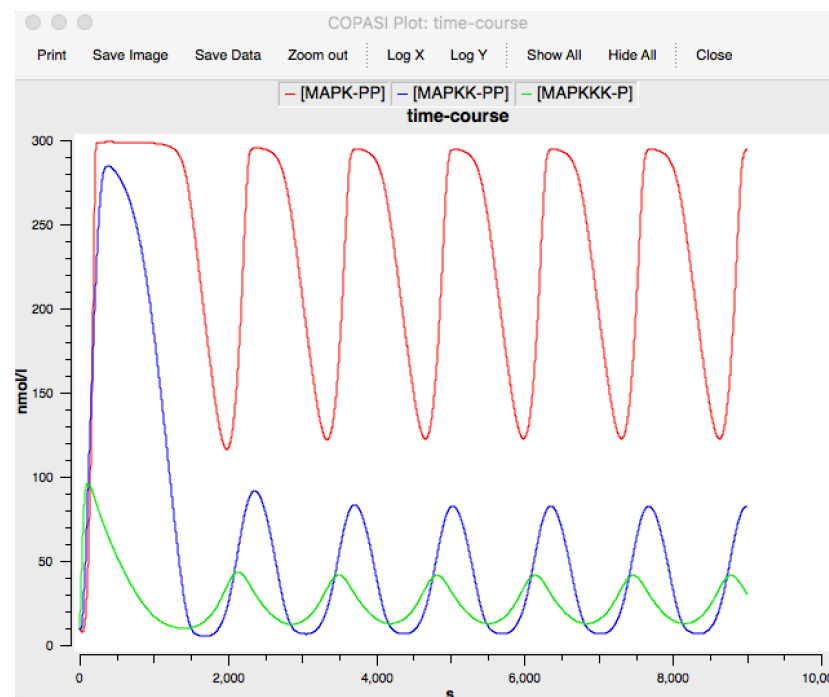
Ki = 18.45

Ki = 46.131

BIOMD0000000027 - Markevich2004 – double phosphorylation causes bistability

If two modification steps or two de-modification steps are catalysed by the same enzyme, bistability can be generated.

This model demonstrates that both dual and multisite modification cycles can display bistability and hysteresis.
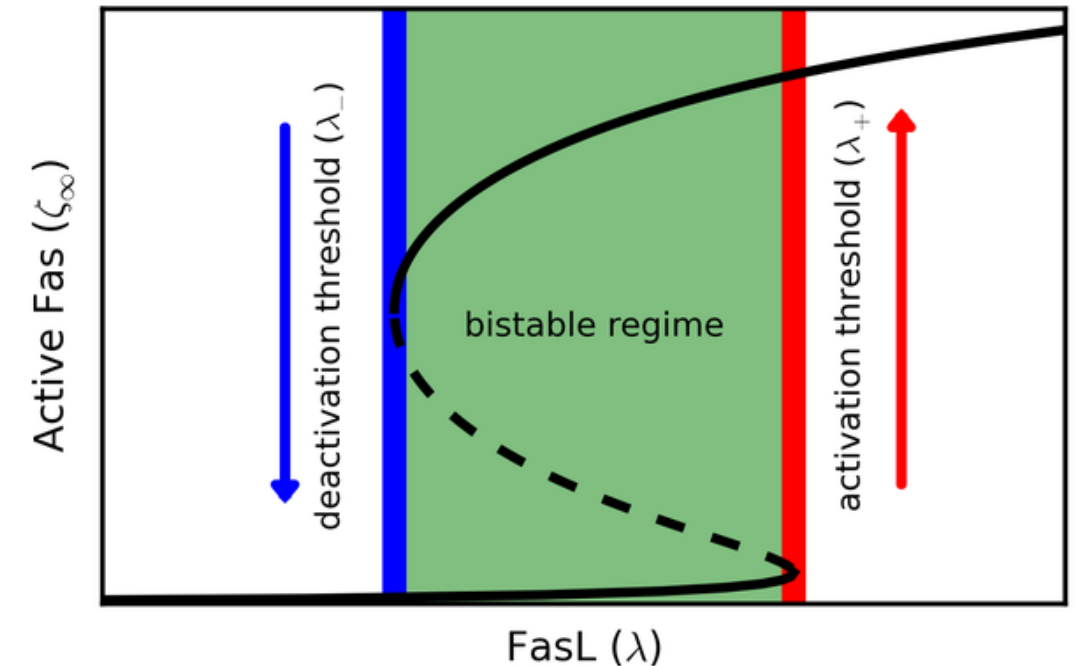
BIOMD0000000027 - Markevich2004 – double phosphorylation causes bistability

Bistability

Over a certain region of signal, a system shows two steady states.

The system can switch state, either if the signal goes over the activation or fall under the deactivation threshold.

The system exhibits a memory effect, also known as hysteresis

Kenneth L. Ho, Heather A. Harrington: Bistability in Apoptosis by Receptor Clustering; PLos Comp. Biol. 2010 6(10): e1000956.

BIOMD0000000027 - Markevich2004 – double phosphorylation causes bistability

Stimulus strength (MAPKK from 0 to 100) against MAPK activity:

MAPK activity works like a switch with a memory: i.e. MAPK activity not only depend on the stimulus strength (MAPKK), but also on the prior state of MAPK (hysteresis).

Dependence of bistability on Km1:

The size of the bistable region depends on the value of Km1. Further analysis of the system showed that Km1 has to be smaller than Km2 i.e. the first phosphorylation step has to saturate at much lower concentrations of M than the second.