Babraham Institute

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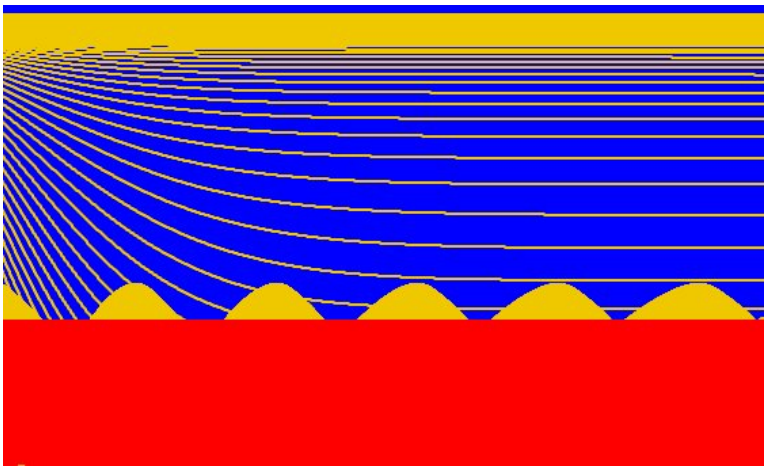
Building and Simulating Models using COPASI

12 July 2017



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Introduction

This tutorial is not meant to be an advanced course. However, it aims at being practical and after completing it you should be able to create your own simple models and run a few simulations and analyses. There are many modelling approaches, and many model analysis procedures. We will only concentrate on one type of model: Chemical kinetics in well mixed solutions. Such models have been around since the mid 20th century and are still the most used type in systems biology.

We will use a mix of models that were actually published as part of research projects, and toy models specially created for the sake of this tutorial. Their size and complexity have been chosen to fit the duration of the tutorial.

Many concepts will be introduced and not developed. For more general background on the modelling of biochemical networks, see:

- Le Novère. Quantitative and logic modelling of gene and molecular networks. *Nature Reviews Genetics* (2015) 16: 146–158

For a complete introduction to systems biology, including modelling, see:

- Edda Klipp *et al*. Systems Biology: A Textbook. *Wiley* (2016)

A good book focussed on modelling, with tutorials, is:

- Fall *et al*. Computational Cell Biology. *Springer* (2002)

Finally, the following book presents different approaches used to model dynamic phenomena in life sciences, expanding further than the previous ones. It comes with plenty of exercises in MatLab and R.

- Ellner and Guckenheimer. Dynamic Models in Biology. *Princeton University Press* (2006)

Modelling software

This tutorial will use the modelling and simulation tool COPASI (standing for "**Co**mplex **Pa**thway **Si**mulator"). COPASI 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

Models

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).

The Mitogen Activated Protein Kinase cascade is a prototypic example of behaviour emerging from a system. As we will see, the response of a MAP kinase to an upstream signal is shaped by the whole cascade of successive phosphorylation. The prediction that such a cascade would engender ultrasensitivity is one of the great successes of mathematical modelling in biochemistry, made in 1981 by Albert Goldbeter and Daniel Koshland, a decade before the discovery of MAP kinase cascades. More can be found at:

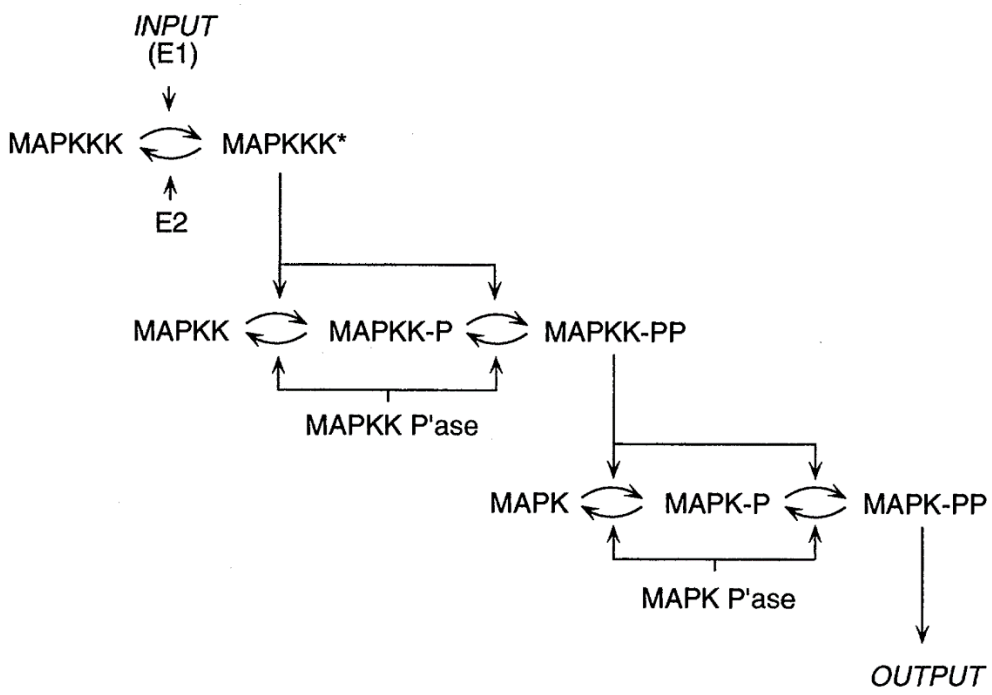
https://nlenov.wordpress.com/2013/07/02/modelling-success-stories-3-goldbeter-and-koshland-1981/

1) MAPK cascade - Ultrasensitivity:

Model:
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)

This was the first detailed model of MAPK signalling showing the effect of cascading phosphorylation steps on MAPK signalling (although as mentioned above, the theory was there already). All intermediate complexes were modelled explicitly with reactions using Mass Action kinetics (compare this approach with the one used in the next exercise). Parameters and concentrations were estimated from experimental results. The rate equations of the cascade were solved numerically.



Schematic view of MAPK cascade (Huang and Ferrell. 1996)

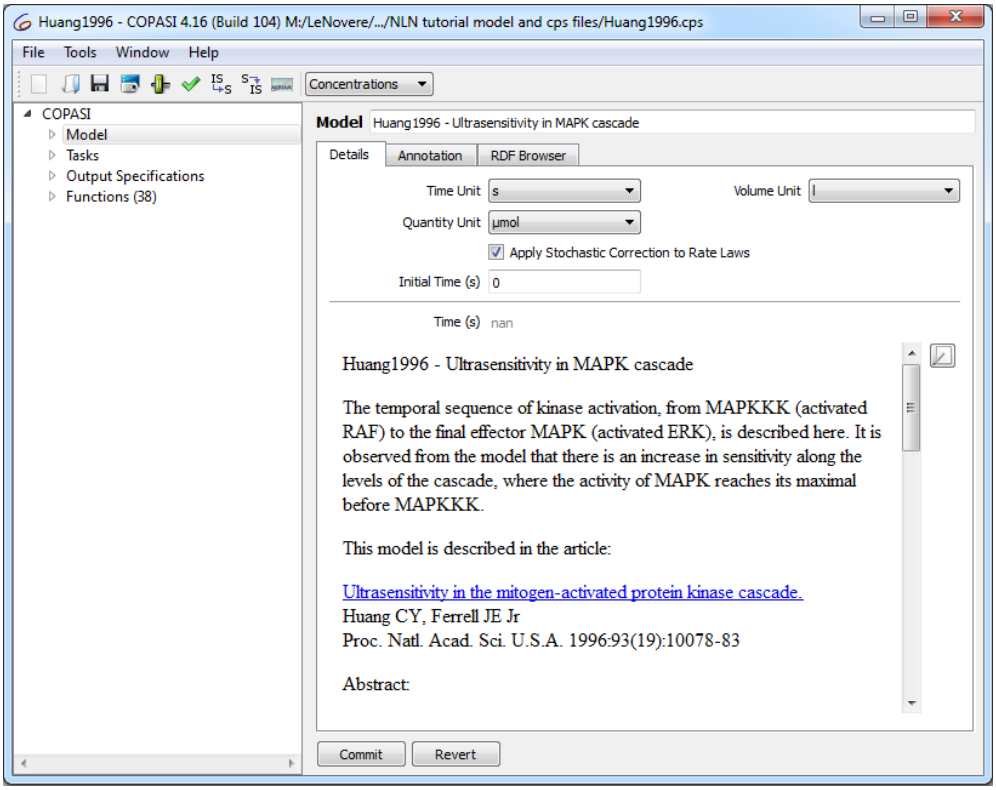
Predicted results were similar to experimental results obtained with *Xenopus laevis* oocytes. In particular, the dose response curves for MAPK, MAPKK, MAPKKK were predicted to show increasing ultrasensitivity (or supra-linearity), with the MAPK curve predicted to be the steepest.

In this exercise, we will import a model already written, using the format SBML (Systems Biology Markup Language, http://sbml.org).

1.a Running a time course simulation:

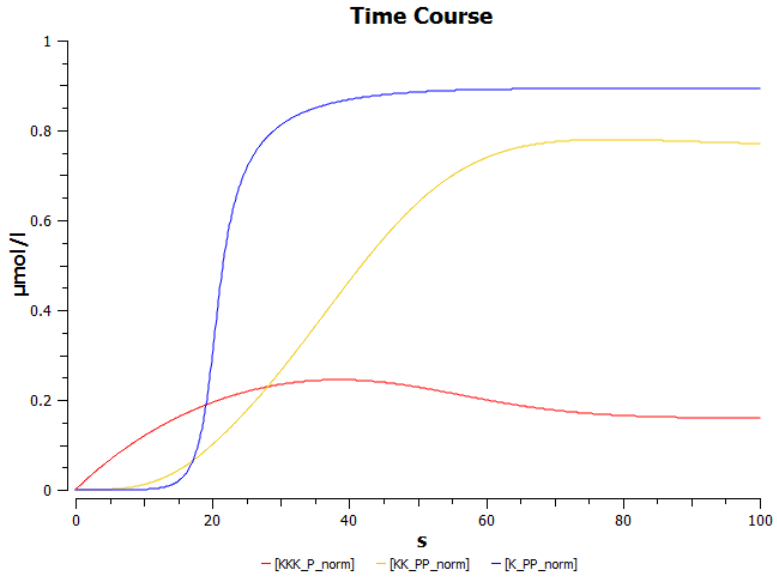
A time course simulation shows the evolution of a system over time. It computes the values of the variables (here the concentrations) after a small amount of time based on their values now. Time courses are particularly important to understand signalling events and their consequences.

- Import **Huang1996.xml** into COPASI.



- Explore the model and try to understand what each reaction does. Use the map on the previous page for context. Look at the “Annotation” tab of “Model”, a selected “Species” and a “Reaction”.
- Prepare a Time Course (Under Tasks) with duration = 100 seconds, Interval Size = 0.1 seconds, Method = Deterministic (LSODA).
- Use the Output Assistant to create a “Concentrations, Volumes and Global Quantity Values” plot, and then run the time course.
- Hide all of the curves except for the normalized active forms of MAPK (“K_PP_norm”), MAPKK (“KK_PP_norm”) and MAPKKK (“KKK_P_norm”).
- Save your model (e.g Huang1996_time_course.cps)

The plot below shows that MAPKKK (e.g. Raf) is activated first, then MAPKK (e.g. MEK) and finally MAPK (e.g. ERK). However, MAPK reaches maximal activation in 30 seconds, well before MAPKK (40 secs) and MAPKKK (70 secs). Why does MAPKKK show a maximum at 40 secs, then decreases to reach a lower plateau?



1.b Creating a dose-response curve – sensitivity to signal:

Dose response studies help us understand any change caused by varying a dosage on the dynamics of the system. These are used for instance to determine the efficient, “safe” and “hazardous” dosages of drugs and optimize any perturbation protocol. The numerical procedure is called parameter scan, or parameter sweep. As the name indicates, they can also be used to study the effect of varying parameters in a model and study the sensitivity of a system towards this parameter (there are more efficient way to do this, which are beyond the scope of this course).

Here, we will study the stimulus/response curves for three components of the MAPK cascade, the input stimulus being the MAPKKK_activator (INPUT(E1) on the map above, for instance Ras). We will compute the steady-state of all concentrations (when their values do not vary anymore) for increasing values of the MAPKKK_activator.

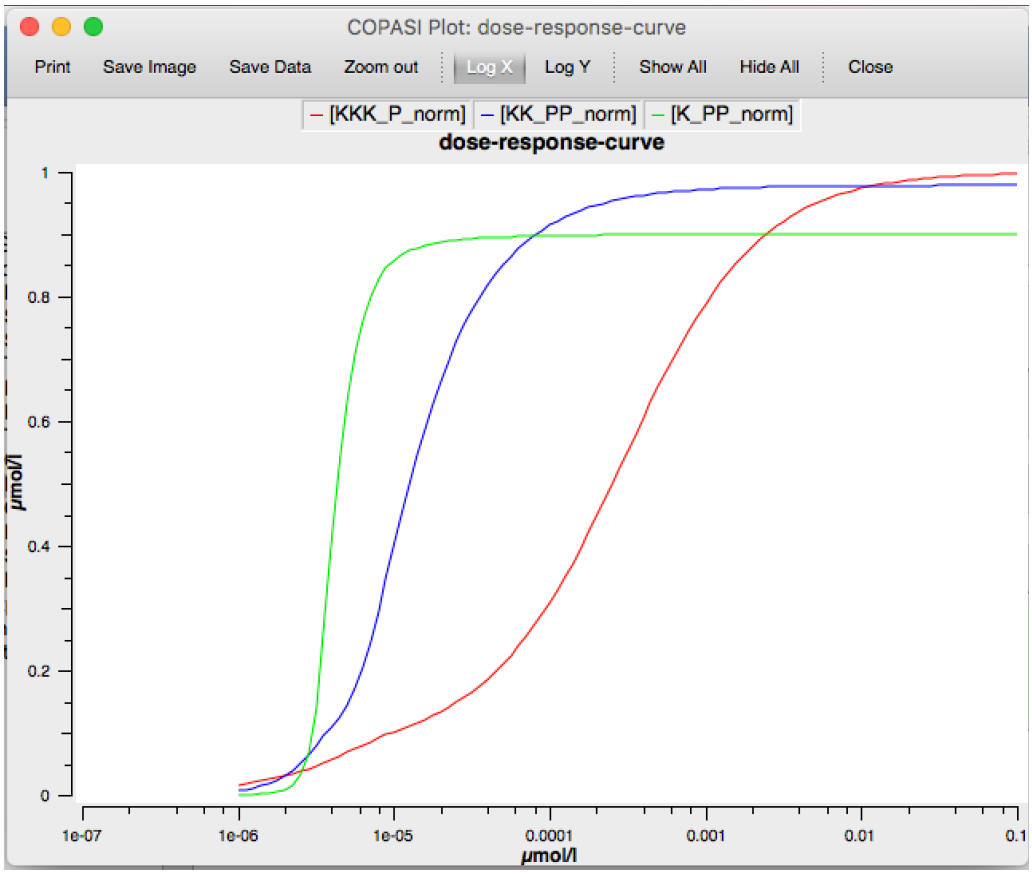
- Select Parameter Scan (Under Tasks), and change the Task type to “Steady State”.
- Set new scan item to Scan and click create.
- Select the parameter to be scanned as Initial Concentration of the [MAPKKK activator](t=0)
- In the scan item you have just created set: Intervals = 100, min = 1e-6, max=0.1, and select logarithmic
- Use the Output Assistant to create a “Scan of Concentrations. Volumes and Global Quantity Values” plot. Use a log scale for the x-axis (MAPKKK_activator). Run the scan to get the dose response curves.
- Hide all of the curves except for the normalized active form of MAPK (“K_PP_norm”), MAPKK (“KK_PP_norm”) and MAPKKK (“KKK_P_norm”).
- Save your model (e.g Huang1996_parameter_scan.cps)

This plot teaches us two different, very important, characteristic of the MAP kinase cascade.

1. The half activation of ERK happens at lower RAS concentration (4.4 µM) than that of MEK (13 µM) or RAF (260 µM).
2. The slope of the curve for ERK is steeper than for MEK, itself steeper than for RAF

Such a cascade of covalent modifications provided a new mechanism to generate ultrasensitivity, in addition to the allosteric regulation of multi-site multimeric proteins. In addition, this is a brilliant demonstration of the emergent properties of a biological system, and the absolute need to study the behaviour of the whole system.

An *in vitro* reconstitution of RAS and RAF would tell us that RAS "EC_{50}" is 260 µM, and its dynamic range (the range of signal giving 10% to 90% of response) of [10 µM, 2.4 mM]. While in fact, if we consider that ERK is the final readout, the "EC_{50}" is 100 fold lower at 4.4 µM, and the dynamic range of [3 µM, 7.4 µM]. That makes the MAP kinase a thresholding system, which transforms a continuous quantity into a binary response, while RAF transmits a quantitative measurement below. NB: ERK on its own would not produce a threshold when stimulated by MEK. The behaviour emerges from the stacking of the three protein activations.



2) MAPK cascade - Oscillations:

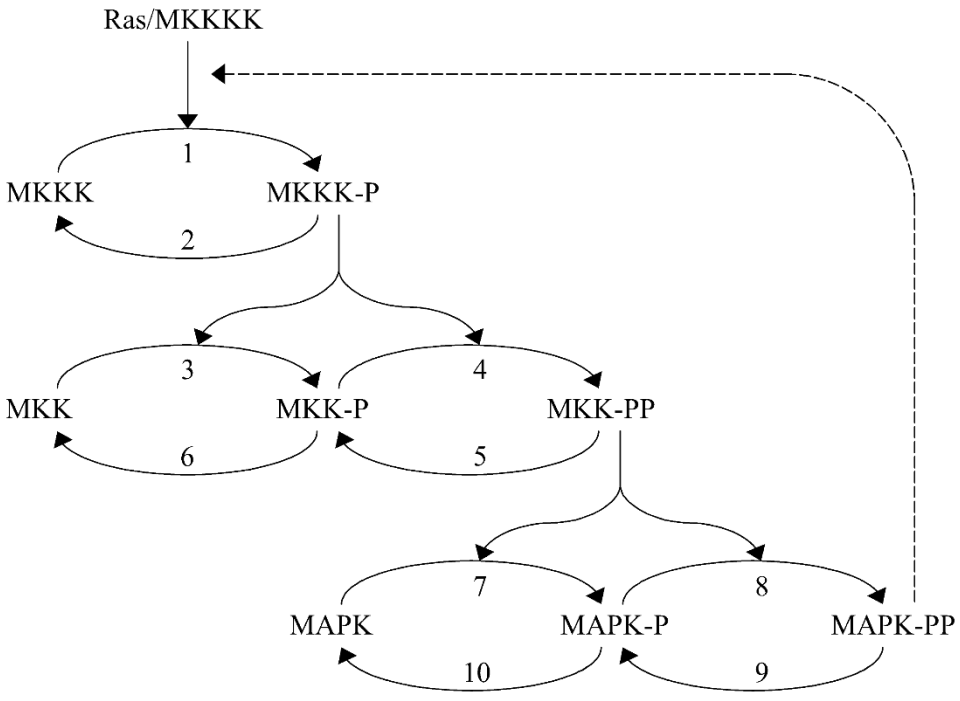
Model:
BIOMD0000000010 – Kholodenko2000

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)

Inhibitory phosphorylation of SOS by p42/p44 MAPK (ERK) provides a mechanism for switching off Ras signalling. This inhibition creates a negative-feedback in the MAPK cascade (below). Indeed, while tyrosine phosphorylated Raf brings ERK activation, ERK mediated inhibition of Raf stimulation by SOS decreases ERK phosphorylation. The paper by Kholodenko attempted to show that a combination of negative feedback with ultrasensitivity can lead to sustained biochemical oscillations.

Here, we will see the effect of negative feedback (MAPK on its activator MAPKKK) on the dynamics of MAPK signalling cascade.

The topology of the reaction network of this model is identical to Huang and Ferrell's model (the model we used in the previous example), apart from the negative feedback. However, for this model, all reactions were modelled as simple Michaelis-Menten kinetics, unlike in Huang and Ferrell model where the reactions are modelled using mass-action kinetics.



Schematic view of MAPK cascade with negative feedback loop. (Kholodenko 2000)

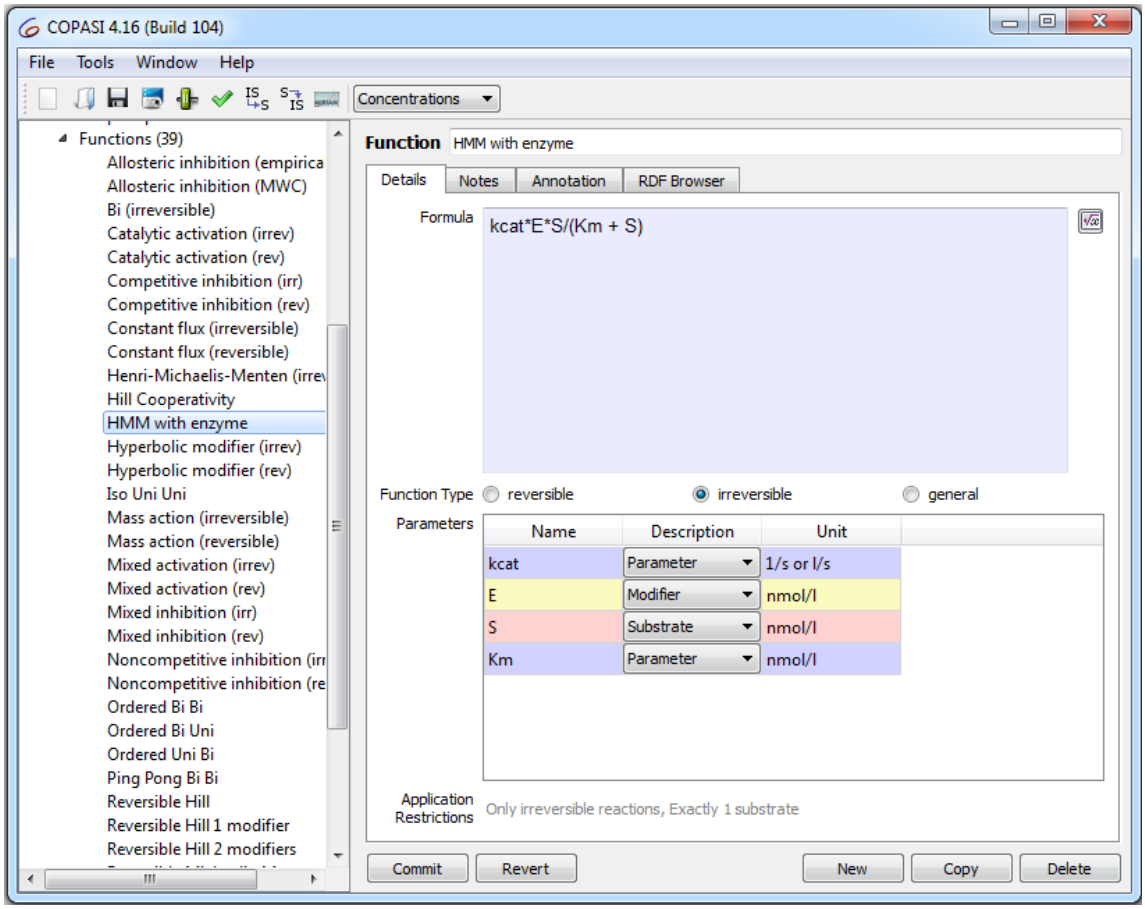
2.a Building the model without the negative feedback:

- Open a new COPASI file, and set the name of the model to “Kholodenko2000-noFeedback”
- Set the units: Time = s, Volume = l, Quantity = nmol
- Create a new compartment (Model>Biochemical>Compartments), name it “cell” and set the initial volume to 1

For the purpose of this tutorial we need the form of the Henri-Michaelis-Menten law that uses k_{cat} and the concentration of enzyme. COPASI only comes with the classic “Michaelis-Menten” law using Vmax.

- Create (and commit) the following function (Under Functions):

  Function : HMM with enzyme
  Formula : kcat*E*S/(Km + S)
  Function type : irreversible
  Parameters : Change the description for S to substrate
  Change the description for E to modifier

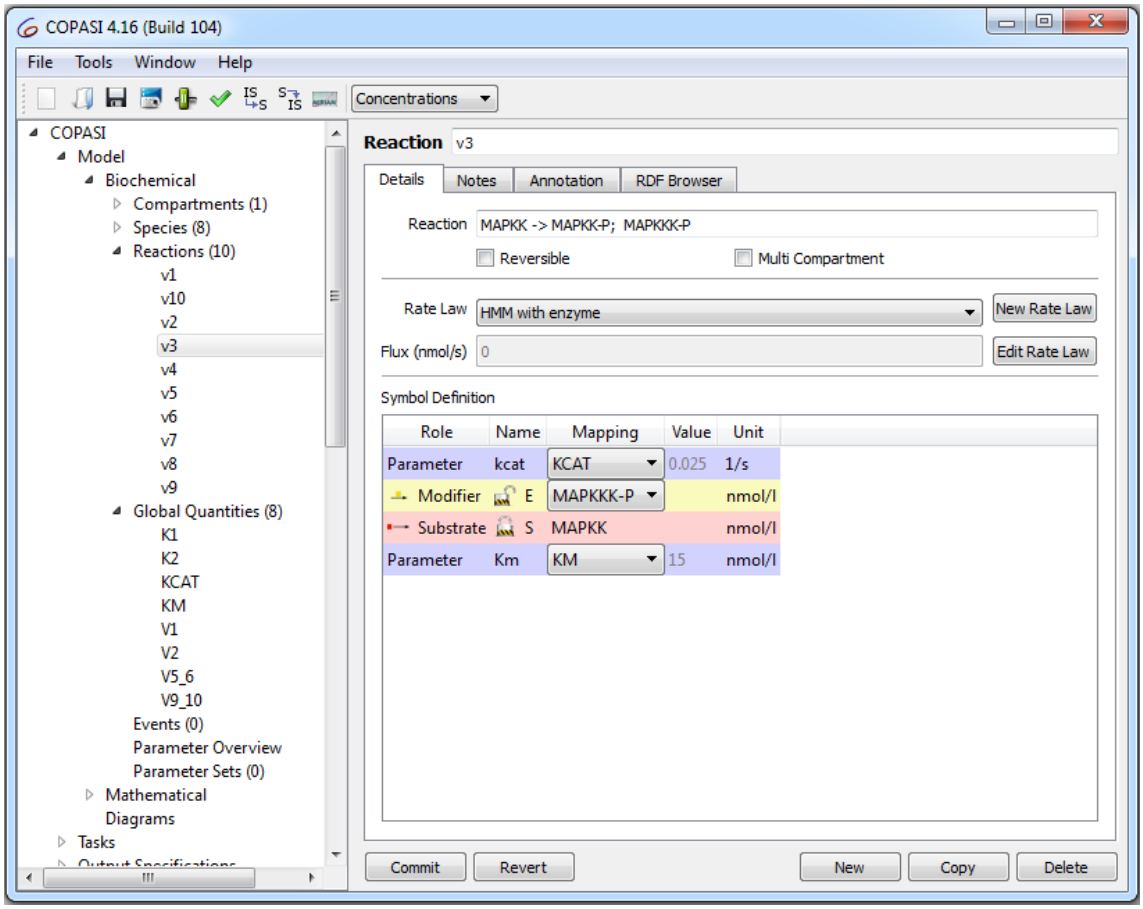


- Create (and commit) the following parameters as Global Quantities (Model>Biochemical>Global Quantities):

Parameter	Initial Value
V1	2.5
K1	10.0
V2	0.25
K2	8.0
KCAT	0.025
KM	15.0
V5_6	0.75
V9_10	0.5

- Add the following reactions (Model>Biochemical>Reactions):
  (HMM = Henry-Michaelis-Menten (irreversible) ; HMMenz = HMM with enzyme)

Name	Equation	Rate Law	Mapping
v1	MAPKKK -> MAPKKK-P	HMM	V → V1; Km → K1
v2	MAPKKK-P -> MAPKKK	HMM	V → V2; Km → K2
v3	MAPKK -> MAPKK-P ; MAPKKK-P	HMMenz	kcat → KCAT; Km → KM; E → MAPKKK-P
v4	MAPKK-P -> MAPKK-PP ; MAPKKK-P	HMMenz	kcat → KCAT; Km → KM; E → MAPKKK-P
v5	MAPKK-PP -> MAPKK-P	HMM	V → V5_6; Km → KM
v6	MAPKK-P -> MAPKK	HMM	V → V5_6; Km → KM
v7	MAPK -> MAPK-P ; MAPKK-PP	HMMenz	kcat → KCAT; Km → KM; E → MAPKK-PP
v8	MAPK-P -> MAPK-PP ; MAPKK-PP	HMMenz	kcat → KCAT; Km → KM; E → MAPKK-PP
v9	MAPK-PP -> MAPK-P	HMM	V → V9_10; Km → KM
v10	MAPK-P -> MAPK	HMM	V → V9_10; Km → KM



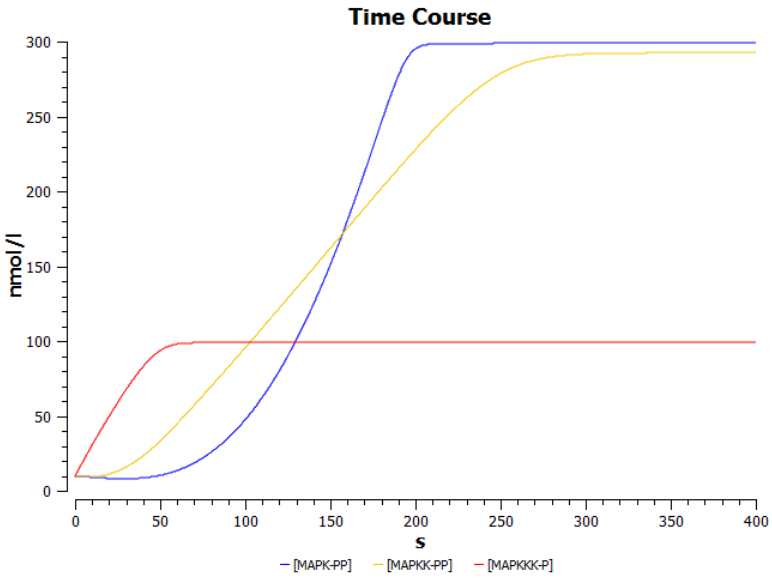
- Set the initial concentrations for the following species:

Species Name	Initial Concentration
MAPKKK	90
MAPKKK-P	10
MAPKK	280
MAPKK-P	10
MAPKK-PP	10
MAPK	280
MAPK-P	10
MAPK-PP	10

- Save your model (e.g Kholodenko2000_noFeedback.cps)

2.b Running a time course simulation with our model:

- Create a time course (as in Exercise 1), with a Duration = 400 secs and Interval Size = 0.1 secs.
- Create a “Concentrations, Volumes and Global Quantity Values” plot and run the time course.
- Show only the curves for the normalized active form of MAPK (MAPK-PP), MAPKK (MAPKK-PP) and MAPKKK (MAPKKK-P).



Do we retrieve the results obtained in 1.a? What is different in the curves representing activated RAF (MAPKKK-P)? What causes the difference?

2.c Introduce negative feedback to the model:

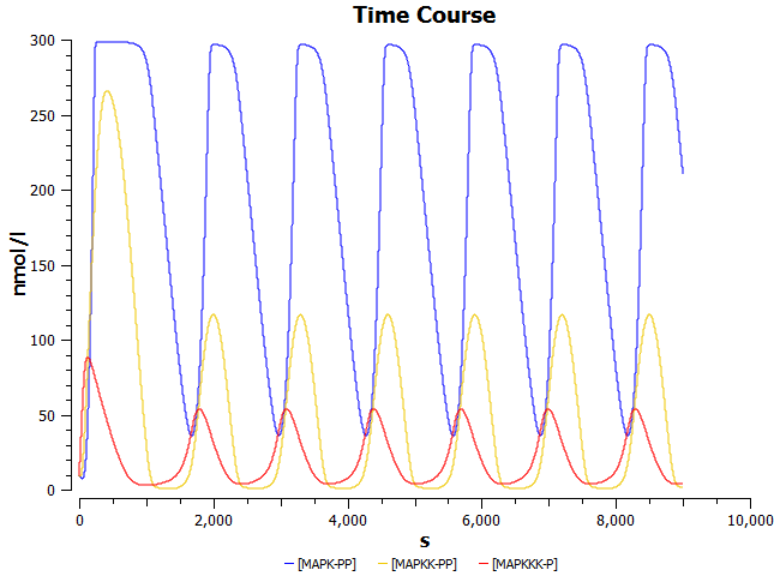
- Create a new function:
  Function : HMM with inhibition
  Formula : V*S/((1+(I/Ki)^n)*(K+S))
  Function type : irreversible
  Parameters : Change the description for S to substrate
  Change the description for I to modifier

- Change the rate law of MAPKKK activation (reaction v1) to the new function “HMM with inhibition”, and set the parameter values as follows (first, set the “mapping” to “--local--”)

Parameter	Value
Modifier	MAPK-PP
V	2.5
Ki	9
K	10
n	1

- Save your model (e.g Kholodenko2000_negFeedback.cps)
- Run a time course as above, with a duration of 9000 seconds

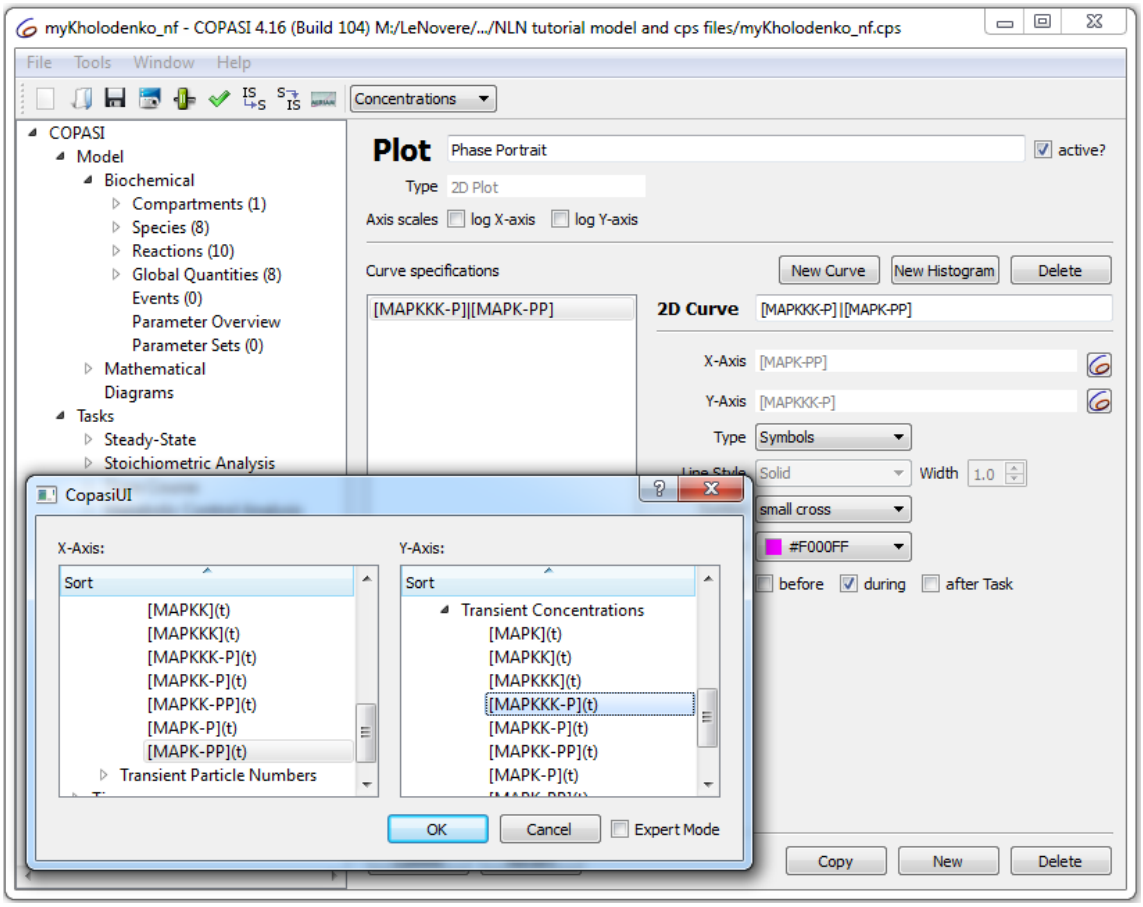
What happens? What causes the dynamical behaviour you observe? Would negative feedback always produce such a result?



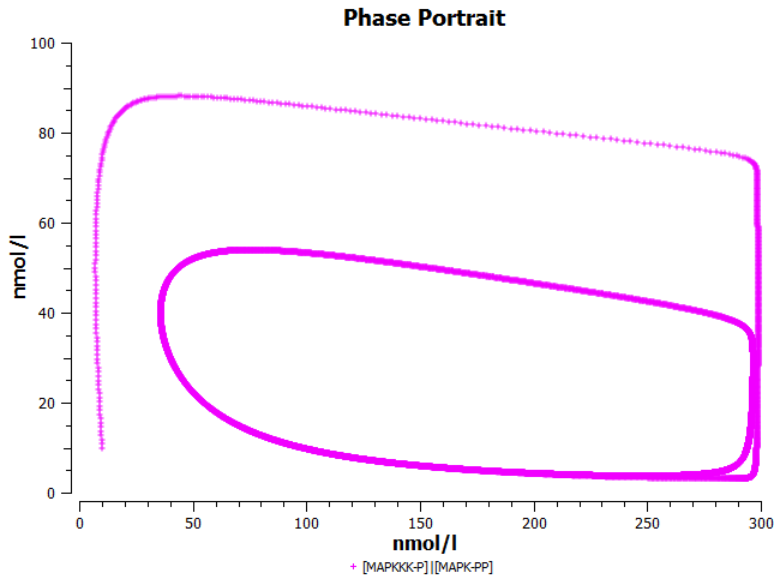
[2.c cont’d - optional/advanced]

Plotting the values of state variables (here concentrations) versus time is not the only way to depict the dynamical behaviour of a system. Other graphical representations can reveal regulatory structures. A plot frequently used to display dynamical behaviours is the phase portrait, where we plot the value of a variable versus the value of another over time.

- Create a plot that reports MAPKKK-P as a function of MAPK-PP. Rerun the simulation.

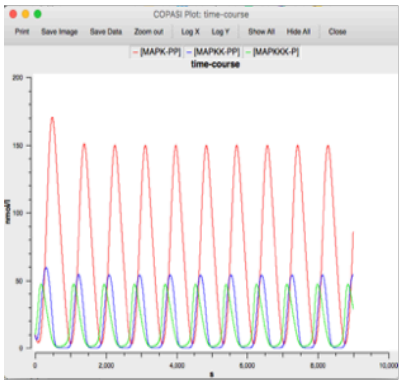


What do you observe? Can you predict what such a plot would look like for a steady state? A damped oscillation? A bi-stable switch?

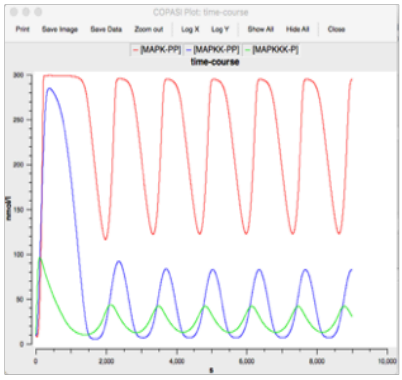


2.d Dependence on inhibition:

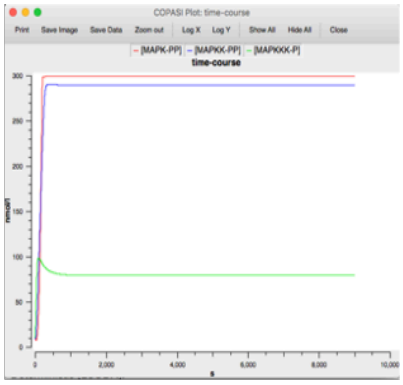
- Navigate to the Time Course Task. From here,
- K_i indicates the concentration of the inhibitor needed for half-maximal inhibition. Create a new **Time Course** slider from the Tools Menu. Sliders in COPASI allow users to change parameter values and generate plots interactively. This can be used to study the effects of inhibition.
- Choose to vary Ki (Reactions>Reaction Parameters>v1(MAPKKK activation)>Ki) (you can try using a logarithm slider between 1 and 100) in the scan and view the plot (you don't need to create new plots - they were already generated in the previous section). The position of the slider shows the current value of Ki. By choosing the **update ranges** and **update automatically** options, and running the task, one can generate time course plots for different values of Ki. (you don't need to create new plots - they were already generated in the previous section).
- Note the changes in the time course, as you increase Ki values.
- the point where the oscillation begins to damp can be observed.



Ki = 1.324



Ki = 18.45



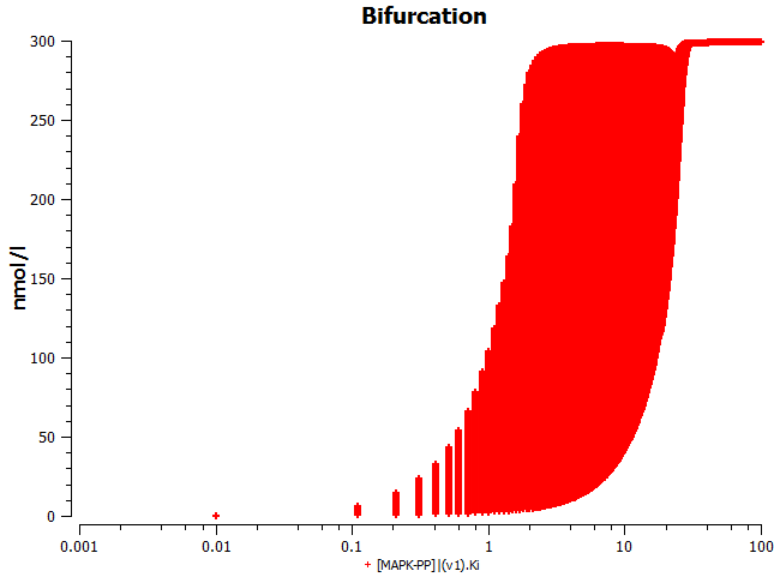
Ki = 46.131

[2.d cont'd -optional/advanced]

The point at which the behaviour switches from reaching steady-state to stable oscillations is called a bifurcation. We can try to pinpoint the respective values of Ki.

- Create a plot "bifurcation", that will display MAPK-PP as a function of reaction v1's parameter Ki. Select a log X-axis, "Symbols" for type and data capture during the tasks.
- Modify the time course to suppress output before 2000 s (I should still stay on a duration of 9000 s. Interval size of 1 are fine.
- Create a **time course parameter scan** exploring logarithmically the values of v1's parameter Ki from 0.01 to 100 (1000 intervals)
- Run the task.

Can you pinpoint the value of Ki for which we start to see big stable oscillations? What happens below? For which value of Ki do the oscillations disappear? What happens above? [look at what happens to the time course. Art and Science …]



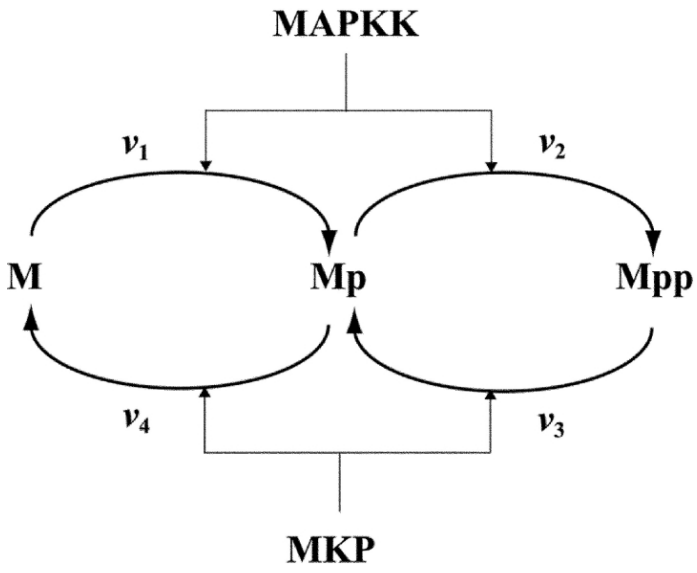
Examples of oscillatory behaviours in MAP kinase cascades have since then be shown experimentally (in https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2602854/
and https://www.ncbi.nlm.nih.gov/pubmed/18384751) .

3 MAPK double phosphorylation (Bistability):

Model
BIOMD0000000027 – Markevich2004 - MAPK double phosphorylation, ordered Michaelis-Menten

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).

The main mechanism by which signals flow along pathways is the covalent modification of proteins by other proteins. In two-step modification enzyme cycles, if The figure below shows a two-step modification enzyme cycle in which both modifier and demodifier enzymes, MAPKK and MKP, follow a strictly ordered mechanism.



Dual phosphorylation–dephosphorylation cycle of MAPK, in which both MAPKK and MKP follow distributive ordered kinetic mechanisms. M, Mp, and Mpp stand for the unphosphorylated, monophosphorylated, and bisphosphorylated forms of MAPK. Reactions v1 and v2 are catalysed by the MAPKK, and reactions 3 and 4 are catalysed by MKP.

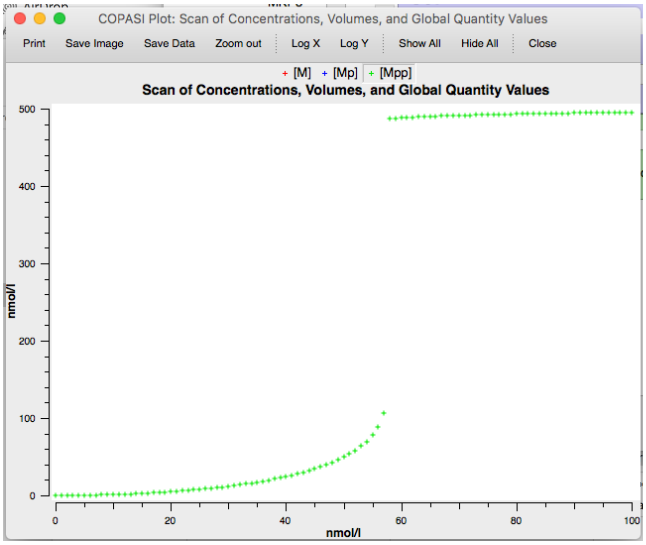
As illustrated in the kinetic diagram, M denote MAPK (eg. ERK1/2) and it has three different forms: M (unphosphorylated), Mp (single phosphorylated) and Mpp (double phosphorylated). MAPKK denote MAP Kinase Kinase (eg. MEK1/2); MKP denotes a dual specificity MAP Kinase Phosphatase (eg. MKP3). Phosphorylation by MAPKK leads to activation, dephosphorylation by MPK leads to deactivation of MAP Kinase (M, Mp, Mpp). The phosphorylation reactions, v1 and v2 are catalysed by MAPKK, the dephosphorylation reactions, v3 and v4, by MKP. All reaction are modelled using irreversible Michaelis Menton kinetics with inhibition by competing substrates. Both M and Mp – but not Mpp – compete for MAPKK, and M, Mp and Mpp bind to and compete for MKP.

3.a Parameter scan to observe the bistable behaviour and hysteresis (Multisite phosphorylation can cause bistability and hysteresis):

Import **BIOMD0000000027.xml** to Copasi (File->Import SBML) and do the following:

Set initial concentration of Mpp from 0 to 500-[M]_0-[Mp]_0 (conservation of total MAPK concentration) by setting an initial expression as follows. Running a parameter scan to see the bistable behaviour and the hysteresis:

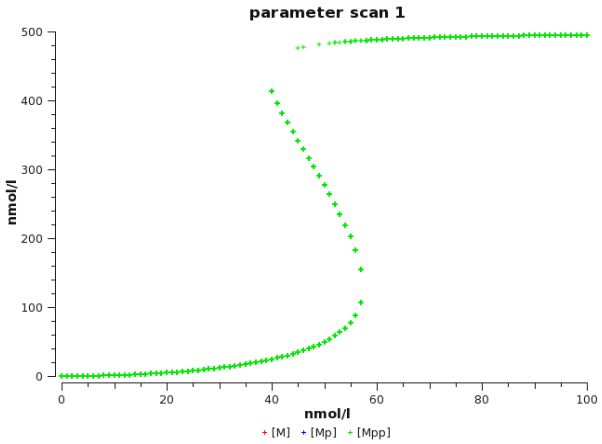
Run a **steady state parameter scan** to vary the initial concentration of MAPKK (MAPKK](t=0) from 0 to 100. Set the intervals to 100 and choose **symbols** when specifying the properties of the curves in the output plot. What do you observe? You don't see the unstable steady states, right?



Can this system exhibit hysteresis?

Hysteresis is the behaviour exhibited by some dynamical systems where a system variable can assume more than one value depending on the direction in which the value of the control parameter generating the variable is varied.

To test this, choose [M](t=0) and add an additional parameter scan of 20 intervals from 100 to 450, to the existing parameter scan. Run the parameter scan.

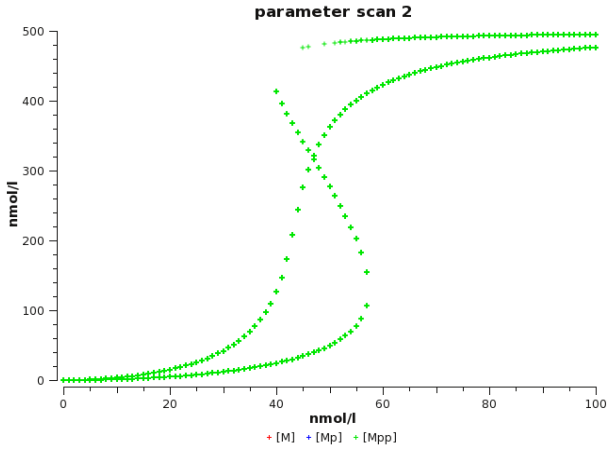


From the plot you will also see that Mpp can have two different values between [MAPKK_P] (t=0) values of 47 to 57. This is a hysteretic region for the system.

Why is bistability important? What is the significance of hysteresis in biological systems?

3.b Significance of Km1 on bistability

To test dependence of bistability on Km1, carry out a **steady state parameter scan** by varying Km1 from 50 to 100 with an interval size of 1. What do you observe from the resultant plot



What is the significance of the bistable region? What does understanding the role of Km1 on bistability reveal about our understanding of the phosphorylation reactions occurring in the pathway.