

Building and Simulating Models using Copasi

WTAC: In Silico Systems Biology
EMBL-EBI, Cambridge, UK
25th April 2012

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Files and supporting materials are available at:
http://www.ebi.ac.uk/~viji/BIOMD-tutorial/WTAC_25April2012/

Download Copasi last stable version 4.8 (Build 35) from:
http://www.copasi.org/tiki-index.php?page_ref_id=106#stable

Exercises:

1. MAPK cascade - Ultrasensitivity :

➤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>)

2. MAPK cascade - Oscillations :

➤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>)

3. Bistability:

➤Markevich NI, Hoek JB, Kholodenko BN. Signaling 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>).

MAPK cascade:

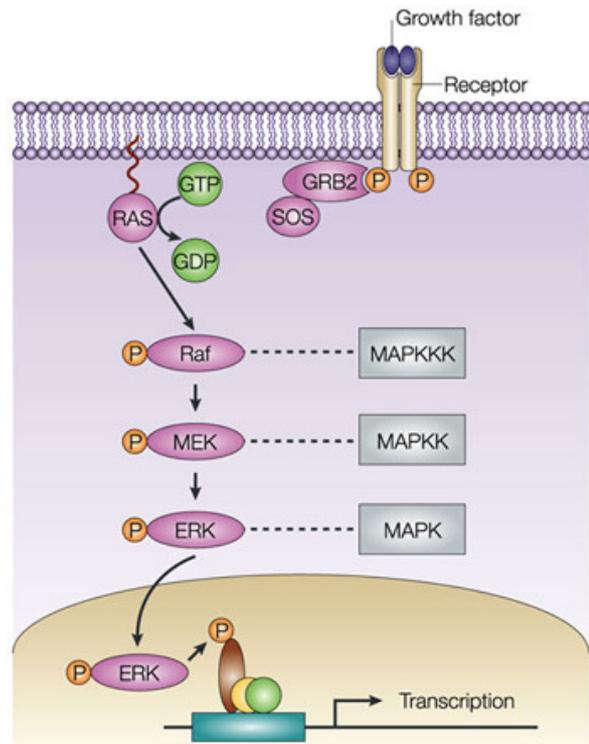
Mitogen activated protein kinase (MAPK) cascades are ubiquitous and highly conserved signalling modules, found in almost all eukaryotes. MAPK cascades are involved in many cellular processes such as cell proliferation, differentiation, movement, survival etc.

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 signaling.



Nature Reviews | Molecular Cell Biology

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

1. MAPK cascade - Ultrasensitivity:

➤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 model demonstrates that the cascade arrangement has unexpected consequences for the dynamics of the MAPK signalling (Figure 2). The rate equations of the cascade were solved numerically. The dose response curves for MAPK, MAPKK, MAPKKK are predicted to be sigmoidal (ultrasensitive), with MAPK curve predicted to be the steepest. Parameters and concentrations were roughly estimated from experimental results. The predicted results were similar to experimental results obtained with *Xenopus laevis* oocytes. In this model all intermediate complexes were modelled explicitly using Mass action kinetics.

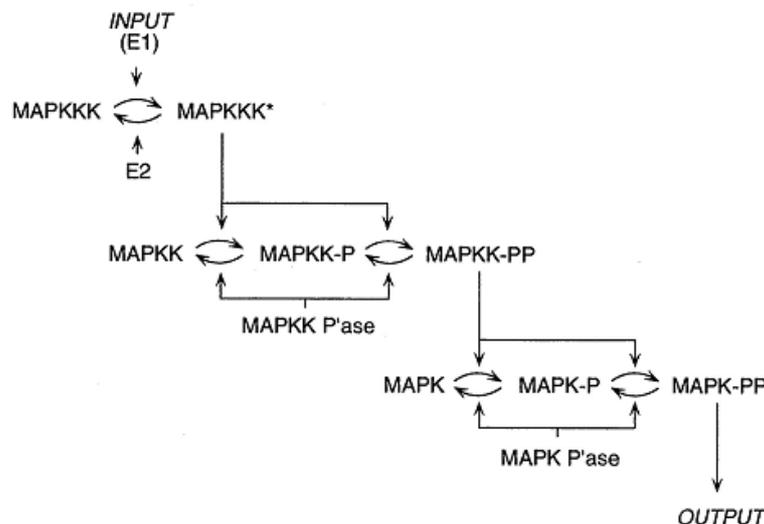


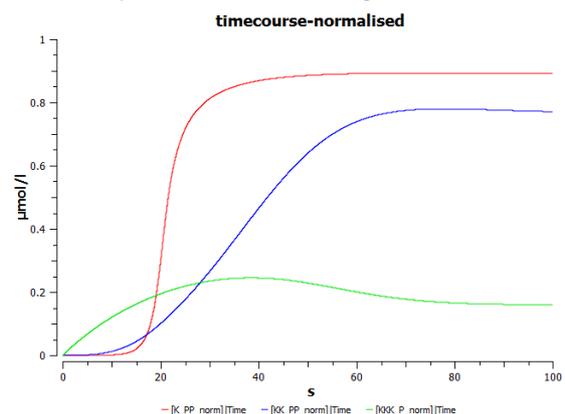
Figure 2: Schematic view of MAPK cascade.

Simulation:

Import **BIOMD0000000009.xml** to COPASI (File->Import SBML) and do the following:

- plot the normalized active form of MAPK (K_PP_norm), MAPKK (KK_PP_norm) and MAPKKK (KKK_P_norm) as a function of time. (Output Assistant->Concentrations, Volumes, and Global Quantity Values).
- run a time-course simulation with a duration of 100 seconds, using a deterministic algorithm. (Tasks->Time Course).

Note: Alternatively, open the file, **huang_timecourse.cps** in COPASI and run time-course to get the plot.



To create a dose-response curve of the active form of MapK against “MAPKKK activator (E1)”:

Create output plot:

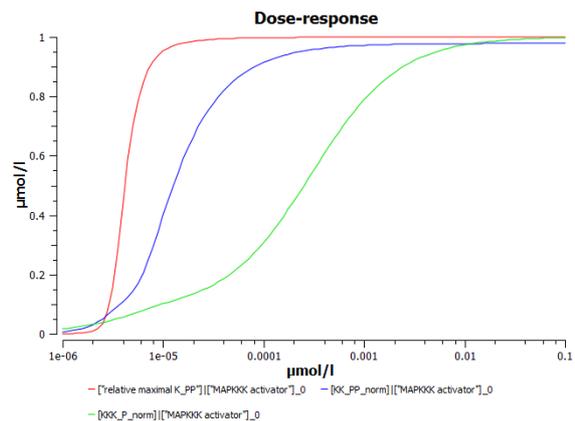
- expand the option “Output Specifications” in the left-hand side menu.
- click on Plots
- select the tab “New” and double click on the newly generated plot.
- click “New curve”
- select Species->Initial Concentrations-> [MAPKKK_activator](t=0) for the

x-axis (left)

- select Species->Transient Concentrations-> [relative maximal K_PP](t) for the y-axis (right)
- do the same for [KK_PP_norm](t) and [KKK_P_norm](t) (hold ctrl to select multiple species)
- in Axes scales check “log X-axis”.
- click commit (as long as the plot is ticked active, it will be updated after every calculation).
- you can deactivate the time course plot for the following exercise.

Sensitivity to signal (dose-response curve to see ultra sensitivity):

- click on Tasks->Parameter Scan
- create a new scan to simulate varying signal strength by varying [MAPKKK_activator]_0 (E1) from 10^{-6} to 0.1 in a logarithmic fashion for 100 intervals as follows:
- New Scan Item: Parameter Scan
- click Create
- in the blue box Scan, under Parameter choose: Species->Initial Concentrations-> [MAPKKK_activator](t=0)
- set Intervals: 100; min:1e-6; max: 0.1
- in the green box Task, choose “Steady State” to plot only the resulting concentration at time- $>\infty$.
- deselect “always use initial conditions” and “output during subtask execution”.
- press Run.



Note: Alternatively, open the file **huang_parameterscan.cps** in COPASI and run parameter scan to get the plot.

Observations:

- The simple time course simulation shows the expected temporal sequence of kinase activation, from MAPKKK to the final effector MAPK. It shows that the activity of MAPK reaches its maximal level before MAPKKK and also hints at the increase in sensitivity along the levels of the cascade.
- The dose-response plot directly shows the strong increase in sensitivity along the levels of the cascade with the MAPK curve predicted to be the steepest.

2. MAPK cascade - Oscillations:

➤ 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 (Figure 3). Indeed, while tyrosine phosphorylated Raf brings ERK activation, ERK mediated inhibition of Raf stimulation by SOS decreases ERK phosphorylation. A combination of negative feedback with ultrasensitivity can lead to sustained biochemical oscillation.

The topology of the reaction network of this model, is identical to Huang and Ferrel's model, apart from the negative feedback. For this model all reactions were modelled as simple Michaelis Menten Kinetics.

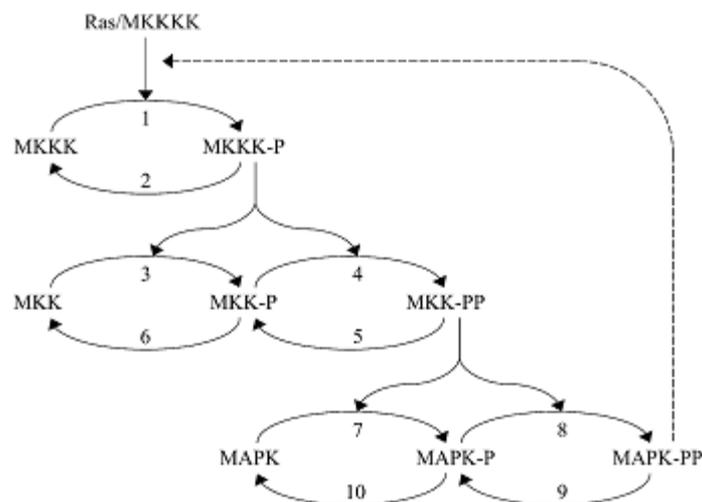


Figure 3: Kinetic scheme of MAPK cascade with negative feedback loop.

Implementing the Model without the negative feedback:

- Create a new model (reopen Copasi or click on File->new or Ctrl+n):
 - In the object tree (left side panel) click on "Model", change name to "Kholodenko2000", set the units to: time – s; volume – l; quantity – nmol.
 - go to Model-Biochemical-Compartments & create a new compartment "cell".
 - press "New" on lower border of the view and edit panel (right hand side).
 - either double click the new entry or click once on each field that you want to alter.
 - set name to cell and initial volume = 1
- Create the following function (under "Functions" click new, go to the second to last function in the list and double click on it):
 - Function name : MM with enzyme
 - Formula : $k_{cat} * E * S / (K_m + S)$
 - Function type : irreversible
 - Parameters : change description for S to substrate;
change description for E to modifier.
- Create the following parameters (under Model->Biochemical->Global Quantities, click new):

Name	Initial Value
V1	2.5
K1	10.0
V2	0.25

K2	8.0
k_cat	0.025
Km	15.0
V5_6	0.75
V9_10	0.5

- Add reactions with kinetic law, reactants, products and modifiers
Under Model->Biochemical->Reactions click new for each reaction. You can copy paste the chemical equation. For the mapping of the parameters, tick "global" and then choose the corresponding parameter from the drop down menu.
(HMM = Henry-Michaelis-Menten (irreversible); MM_enz = MM with enzyme)

Name	Chemical 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	MM_enz	Kcat→k_cat; Km→Km; E→MAPKKK-P;
v4	MAPKK-P -> MAPKK-PP ; MAPKKK-P	MM_enz	Kcat→k_cat; 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	MM_enz	Kcat→k_cat; Km→Km; E→MAPKK-PP;
v8	MAPK-P -> MAPK-PP ; MAPKK-PP	MM_enz	kcat→k_cat; 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

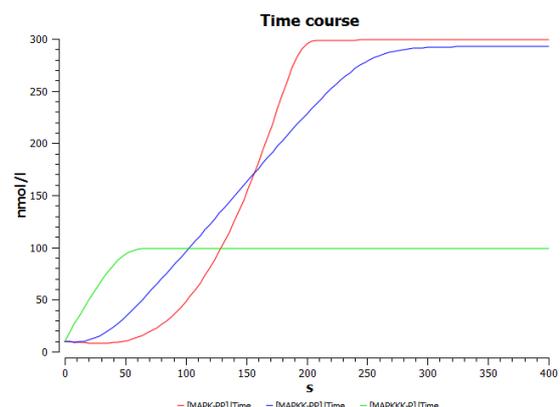
- save the file as **mykholodenko.cps**

Simulation:

Open **kholodenko.cps** (or your own **mykholodenko.cps**) in COPASI and do the following:

- run a time-course simulation of 400 seconds, using deterministic algorithm. (Tasks->Time Course).
- plot the active forms of MAPK (MAPK-PP), MAPKK (MAPKK-PP) and MAPKKK (MAPKKK-P) as a function of time.

Note: Alternatively, open the file **kholodenko_timecourse.cps** in Copasi and run time-course to get the plot.



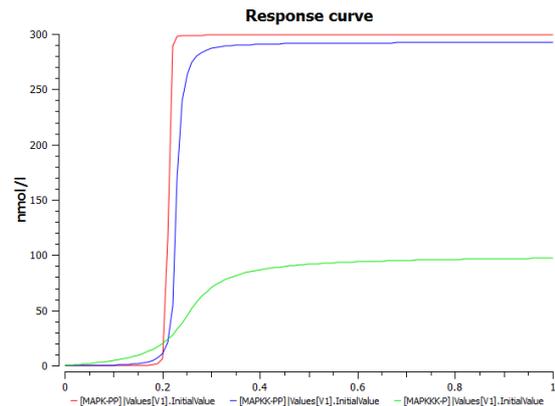
Sensitivity to signal:

Perform the following parameter scan to simulate varying signal strength.

- open **kholodenko.cps**

- vary V1 (Global Quantities->V1) between 0.001 and 1 with 100 intervals.
- choose subtask “Steady State” to plot only the resulting concentration at time-> ∞
- plot the active forms of MAPK (MAPK-PP), MAPKK (MAPKK-PP) and MAPKKK (MAPKKK-P) at steady state as a function of the initial activity of Ras (V1).

Note: Alternatively, open the file **kholodenko_parameterscan.cps** in COPASI and run parameter scan to get the plot.



Negative feedback:

- Replace the kinetic law describing MAPKKK activation (v1) by the following: (add a new function in **kholodenko.cps**).
 Function name : HMM with inhibition
 Formula : $V1 * MAPKKK / ((1 + (MAPK_PP / K_i)^n) * (K1 + MAPKKK))$
 Function type : irreversible
 Parameters : change description for MAPKKK to substrate
 change description for MAPK_PP to modifier
- Then replace the rate law “Henri-Michaelis-Menten (irreversible)” of MAPKKK activation by the new function “HMM with inhibition” and change the parameter values for V1, K_i, K₁ and n (see below).

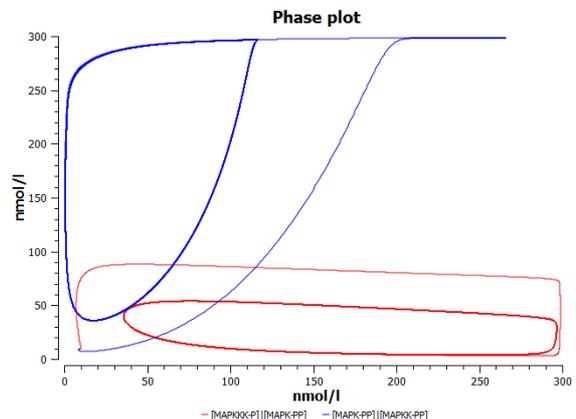
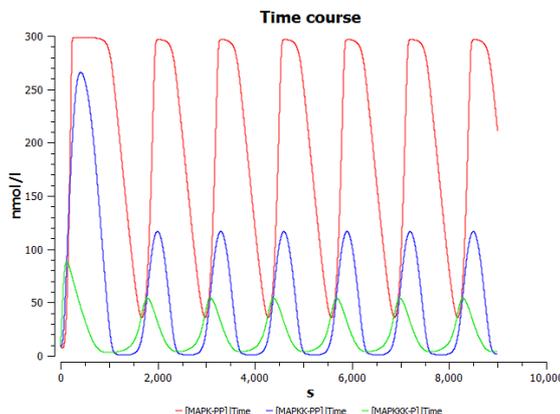
MAPKKK activation:

$$MAPKKK \xrightarrow{MAPK-PP} MAPKKK - P;$$

$$J_0 = \frac{V1 \times [MAPKKK]}{\left(1 + \left(\frac{[MAPK-PP]}{K_i}\right)^n\right) \times (K1 + [MAPKKK])} ; V1 = 2.5 \quad K_i = 9 \quad K1 = 10 \quad n = 1$$

Note: If you have not added the new function definition “HMM with inhibition” as above, in **kholodenko.cps**, then as an alternative open **kholodenko_nf.cps** or import **BIOMD000000010.xml** (which has the negative feedback already added).

- Perform a simulation as above but for a time period of 9000sec and plot the active form of MAPK (MAPK-PP), MAPKK (MAPKK-PP) and MAPKKK (MAPKKK-P) as a function of time (plot in the left side – Time course). Combination of ultrasensitivity and negative feedback brings sustained biochemical oscillations.
- Plot the active form of MAPKKK as a function of the active form of MAPK and the active form of MAPK as a function of the active form of MAPKK (plot in the right side – Phase plot).



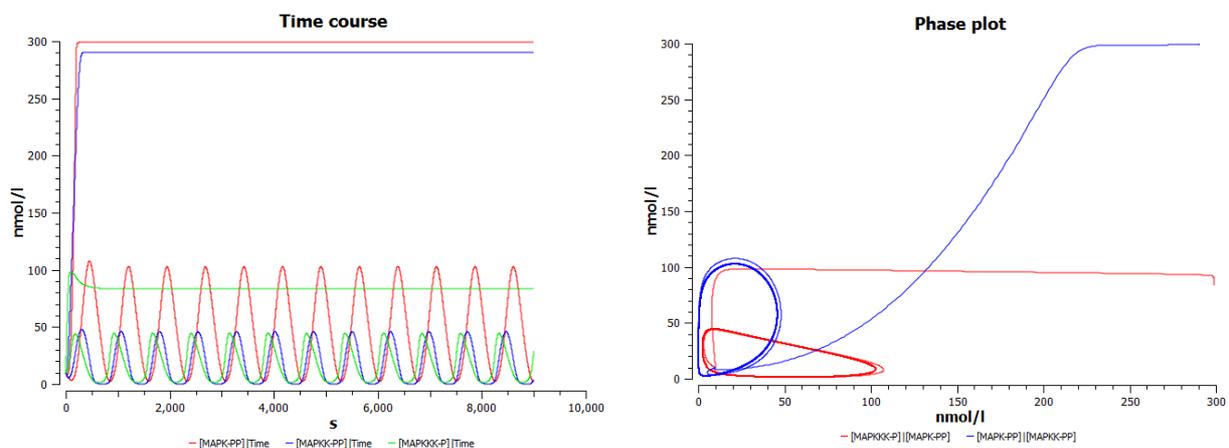
Note: Alternatively, open the file **kholodenko_nf_timecourse.cps** in COPASI and run time-course to get the above plots.

Dependence on inhibition:

Perform the following parameter scan:

- Vary K_i (Reactions->Reaction Parameters->v1 (MAPKKK activation)->Ki) from 1 to 51 in a linear fashion (start with one interval). This simulates a decrease of the inhibitory feedback (K_i indicates the concentration of the inhibitor needed for half-maximal inhibition).
- select Task->Time course.
- press Run (you need not create new plots as they are already generated in the previous section).
- Note the changes in the time course and phase plot when compared to the simulation results obtained in the previous section.

Note: Alternatively, open the file **kholodenko_nf_parameterscan.cps** in COPASI and run parameter scan to get the plots below.



Observations:

- This model shows similar temporal activation profiles and increase in sensitivity to that of Huang and Ferrell, although it uses a different formalism for the derivation of its rate laws.
- Through the inclusion of a simple negative feedback, the system can exhibit oscillatory behaviour under constant stimulation.
- With smaller inhibition constants, the maximal activation achieved decreases, and the frequency of oscillations increases. The system loses the oscillatory behaviour at values of K_i between 25 and 27.

3. Bistability:

➤Markevich NI, Hoek JB, Kholodenko BN. Signaling 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>).

MAP kinase cascades can function as bistable switches, and even exhibit a memory effect, also known as hysteresis. These behaviours are generally seen coupled to positive feedback mechanisms. In this article the authors demonstrate that a single level of the MAP kinase cascade can be sufficient to exhibit this behaviour, without the need for any explicit feedback. The behaviour stems from the distributive two-site phosphorylation and dephosphorylation mechanism. The concentrations and parameters were taken from measurements and estimated from experimental results, in a physiological range.

For these simulations, we are not interested in the exact temporal evolution of the system, but only in the steady state behaviour of the system.

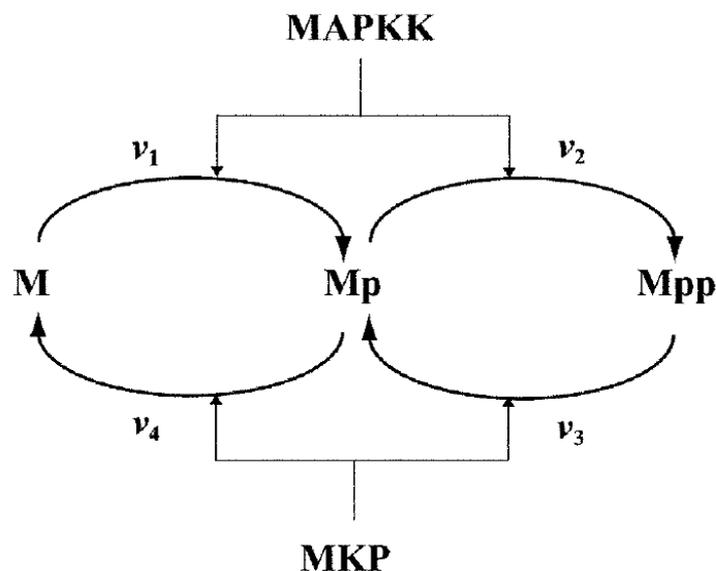


Figure 4: Dual phosphorylation-dephosphorylation cycle of MAPK, in which both MAPKK and MKP follow distributive ordered kinetic mechanism. M, Mp, Mpp stand for the unphosphorylated, monophosphorylated and biphosphorylated forms of MAPK.

The model tries to elucidate the final level of the MAP kinase cascade, as shown in [Figure 4](#). MAPKK denote MAP Kinase Kinase (eg. MEK1/2); MKP denote a dual specificity MAP Kinase Phosphatase (eg. MKP3); and M denote MAP Kinase (eg. ERK1/2). Phosphorylation by MAPKK leads to activation, dephosphorylation by MPK to deactivation of MAP kinase (M, Mp, Mpp). The phosphorylation reactions, v_1 and v_2 are catalysed by MAPKK, the dephosphorylation reactions, v_3 and v_4 , by MKP. All reactions are modelled using irreversible Michaelis Menten 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.

Simulation:

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

Change the initial concentration of Mpp from 0 to 500-[M] 0-[Mp] 0 (conservation of total MAPK concentration) by setting an initial expression as follows:

- select Model->Biochemical->Species->Mpp
- check “use Initial Expression”

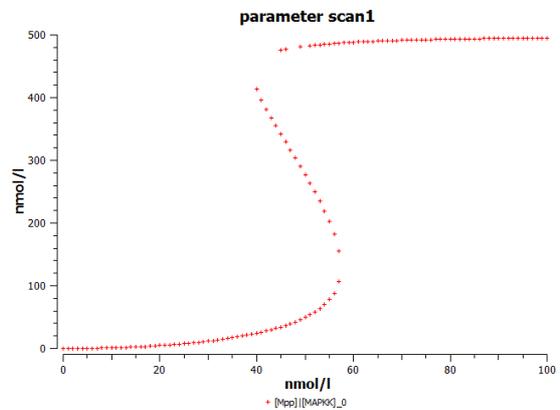
- type the Initial Expression as $500 - [M]_0 - [Mp]_0$ (Note: The species $[M]_0$ and $[Mp]_0$ should be selected one by one by clicking the “Copasi icon” followed by choosing Species->Initial Concentrations-> $[M](t=0)$ and then $[Mp](t=0)$ to get the expression. Numbers and signs can be typed)
- click commit

To create a plot of “MAPKK” against “Mpp”:

- expand the option “Output Specifications” in the left-hand side menu.
- click on Plots
- select the tab “New” and double click on the newly generated plot.
- click “New curve”
- select Species->Initial Concentrations-> $[MAPKK](t=0)$ for the x-axis (left)
- select Species->Transient Concentrations-> $[Mpp](t)$ for the y-axis (right)
- change the curve type to “Symbols”
- click Commit (as long as the plot is ticked active, it will be updated after every calculation).

Running a parameter scan to see the bistable behaviour and the hysteresis:

- go to Tasks->Parameter Scan
- select Task “Steady State”
- under “New scan item:” select “Parameter Scan” and click “create”
- choose the parameter: Species->Initial Concentrations-> $[MAPKK](t=0)$
- do 100 intervals from 0 to 100
- click Run to get the plot.
- add another scan
- choose the parameter: Species->Initial Concentrations-> $[M](t=0)$
- do a scan over $[M](t=0)$ to 20 intervals and from 100 to 450
- press Run

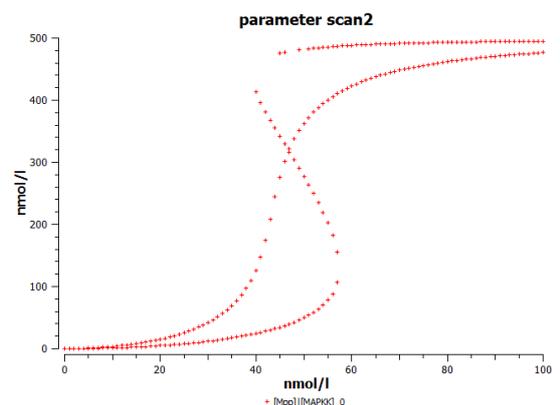


Note: Alternatively, open the file **markevich_parameterscan1.cps** in COPASI, and run parameter scan to get the plot.

Dependence of bistability on Km1:

- add another scan over the global quantity Km1
- do 1 interval from 50 to 500
- press Run

Note: Alternatively, open the file **markevich_parameterscan2.cps** in COPASI, and run parameter scan to get the plot.



Observations:

- At the given parameter values the MAPK activity works like a switch with a memory: MAPK activity depends on the stimulus strength and on the prior state of MAPK.
- 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.