Building and Simulating Models using Copasi

WTAC: *In Silico* Systems Biology
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Exercise - Model Building & Simulation using COPASI:

i. 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)

ii. MAPK cascade – Oscillation:

➢ 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)

iii. MAPK double phosphorylation – Bistability:

➢ 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).
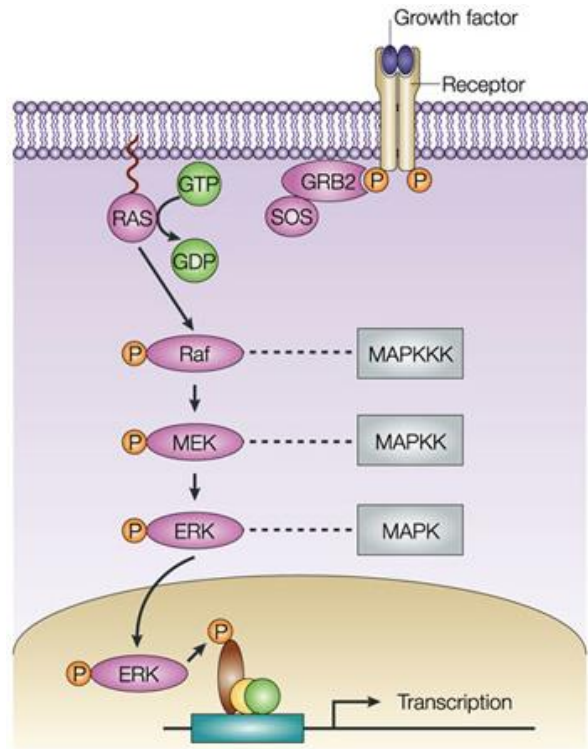
MAPK cascade:

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 signalling.

Nature Reviews | Molecular Cell Biology

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

i. MAPK cascade – Ultrasensitivity:

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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.
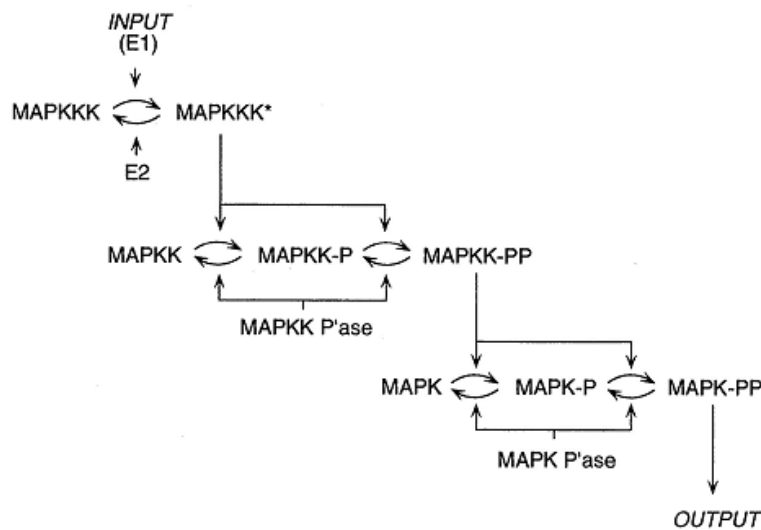

Figure 2: Schematic view of MAPK cascade (Huang and Ferrell. 1996).

To perform a time-course simulation with duration 100 seconds:

- Import **Huang1996.xml** into COPASI (File->Import SBML)
- Go to Tasks->Time Course (left panel)
- On the right panel, Type in 100 in the Duration(s) field and 0.1 in the Interval Size(s) field. Select the Method as deterministic (LSODA).
- To create the plot, click on Output Assistant and choose "Concentrations, Volumes, and Global Quantity Values" in the options displayed and click "Create".
- Click on "Run"
- Plot the normalized active form of MAPK, MAPKK and MAPKKK by selecting "relative maximal K_PP", "KK_PP_norm" and "KKK_P_norm" respectively.
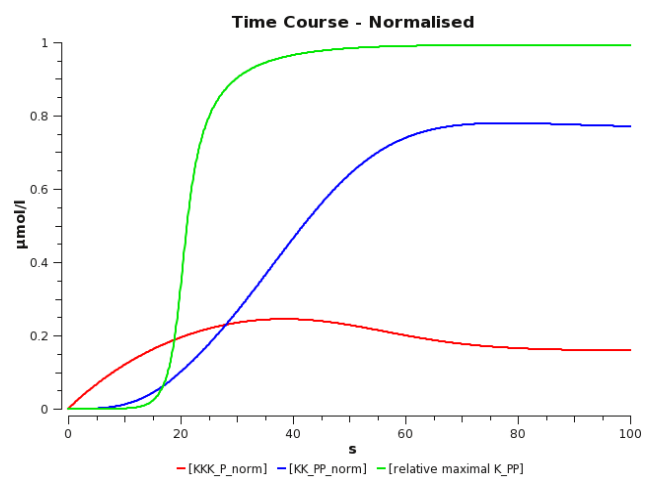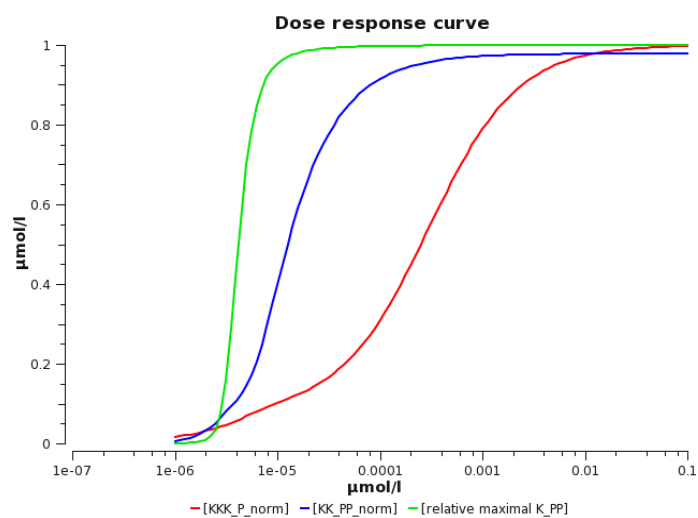

Note: Alternatively, open the file, **Huang1996_timecourse.cps** in COPASI and run time-course to get the plot.

To create a dose-response curve of the active forms of MAPK against "MAPKKK_activator (E1)":

Dose response studies are carried out to understand the effect of variable amount of dose (eg. to determine the "safe" and "hazardous" levels and dosages of drugs) on the dynamics of the systems. Here, we can see the stimulus/response curves for three components of the MAPK cascade, the input stimulus being E1 (MAPKKK_activator). For the x-axis (MAPKKK_activator), log scales are used, to see the exponential increase resulting in Sigmoidal (S-shaped) curves.

Sensitivity to signal (dose-response curve to see ultrasensitivity) by varying [MAPKKK_activator]_0 (E1)

- Go to Tasks->Parameter Scan
- Select Task "Steady State" to plot only the resulting concentration at time->∞
- Under "New Scan Item", select "Scan" and click "Create"
- Choose the parameter that need to be varied, Species->Initial Concentrations-> [MAPKKK activator](t=0])
- set Intervals: 100; min:1e-6; max: 0.1
- check "logarithmic"
- To create the plot, click on Output Assistant and choose "Scan of Concentrations, Volumes, and Global Quantity Values" in the options displayed and click "Create".

- Plot the normalized active form of MAPK (relative maximal K_PP), MAPKK (KK_PP_norm) and MAPKKK (KKK_P_norm) against varying [MAPKKK activator].
- Click on "Run" to get the dose-response curve (observe what happens??).
- In addition, to the stable steady states several unstable steady states are plotted. To just view the stable steady states (as in the figure), set Tasks->Steady-State->Use Newton to "Zero".
- Then go to Tasks->Parameter Scan and Run to get the above plot.

    Note: Alternatively, open the file **Huang1996_parameterscan.cps** in COPASI and run parameter scan to get the plot.

Inference from the time-course and the dose-response curve:

- 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.

ii. MAPK cascade – Oscillation:

> ➤ 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 Ferrell's model (the previous example, BIOMD0000000009), apart from the negative feedback. 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.
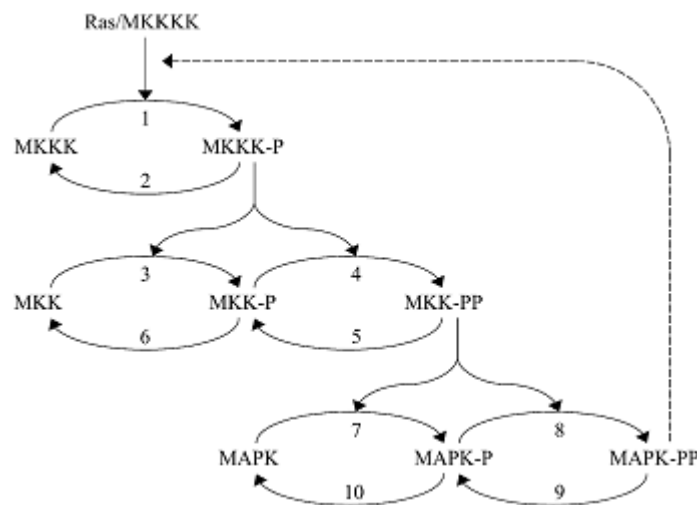

Figure 3: Kinetic scheme of MAPK cascade with negative feedback loop.

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, click on "Model" and set the name of the model as "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 double click on the newly created entry.
    - set Compartment = 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) and "Commit" it:
    - Function : MM with enzyme
    - Formula : kcat*E*S/(Km + 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**

To perform a time-course simulation with duration 400 seconds:

- Open **Kholodenko2000.cps (or your own myKholodenko.cps)** in COPASI.
- Go to Tasks->Time Course (left panel)
- On the right panel, Type in 400 in the Duration(s) field and 0.1 in the Interval Size(s) field. Select the Method as deterministic (LSODA).
- plot the active forms of MAPK (MAPK-PP), MAPKK (MAPKK-PP) and MAPKKK (MAPKKK-P) as a function of time.
- Click on Run to get the plot.

  Note: Alternatively, open the file **Kholodenko2000_timecourse.cps** in Copasi and run time-course to get the plot.
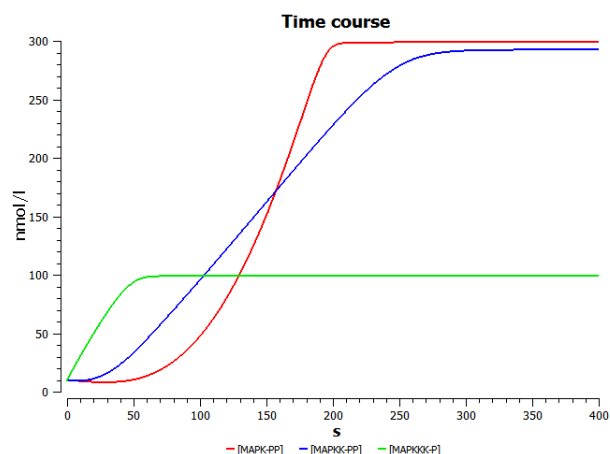
Inference:
- This model shows similar temporal activation profiles and increase in sensitivity to that of Huang and Ferrell, although it uses a different formalism for the derivation of its rate laws.

Observe the behaviour of the system when a negative feedback is introduced:

Feedback loops: The complex biological processes are regulated by feedback loops. In a sequence of event, the effect of the increase (or activation) of a downstream element causes the same effect (i.e. production or activation) to an upstream element, it is termed positive feedback loop. Alternatively, if the increase (or activation) in a downstream element causes a negative effect (decrease or inactivation) to an upstream element, then this process is termed as negative feedback loop. Any disturbances within these feedback loops can be deleterious to the system resulting pathological conditions. In this section, we will see the effect of negative feedback (MAPK on its activator MAPKKK) on the dynamics of MAPK signalling cascade.

- Replace the kinetic law describing MAPKKK activation (v1) by the following: (add a new function in **Kholodenko2000.cps**).

  Function name : HMM with inhibition
  Formula   : V1*MAPKKK/((1+(MAPK_PP/Ki)^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, Ki, K1 and n (see below).

  MAPKKK activation:

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

  $$J0 = \frac{V1 \times [MAPKKK]}{\left(1 + \left(\frac{[MAPK-PP]}{K_i}\right)^n\right) \times (K1 + [MAPKKK])} \quad ; V1 = 2.5 \quad K_i = 9 \quad K1 = 10 \quad n = 1$$

  Note: If you have not added the new function definition "HMM with inhibition" as above, in **Kholodenko2000.cps**, then as an alternative open **Kholodenko2000_nf.cps.**

- 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).

  Note: Alternatively, open the file **Kholodenko2000_nf_timecourse.cps** in COPASI and run time-course to get the above plots.


Time course
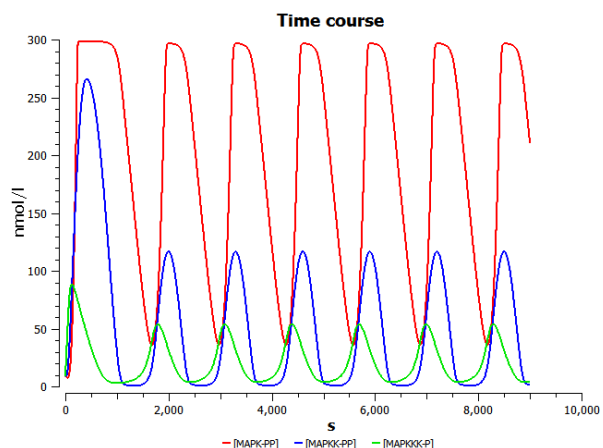
Inference:

- Through the inclusion of a simple negative feedback, the system can exhibit oscillatory behaviour under constant stimulation. i.e. The combination of ultrasensitivity and negative feedback brings sustained biochemical oscillations.

Dependence on inhibition:

Perform the following parameter scan:

- select Tasks->Parameter Scan
- Vary K_i (Reactions->Reaction Parameters->v1 (MAPKKK activation)->Ki) from 1 to 51 in a linear fashion (start with one interval – figure on the left side). This causes a decrease of the inhibitory feedback (K_i indicates the concentration of the inhibitor needed for half-maximal inhibition).
- Click on Run to get the plot (you need not create new plots as they are already generated in the previous section).
- Note the changes in the time course, when compared to that of the plot obtained in the previous section.
- With the interval size of 2 (figure on the right side), the point where the oscillation begins to damp can be observed.

  Note: Alternatively, open the file **Kholodenko2000_nf_parameterscan.cps** in COPASI and run parameter scan to get the plots below.
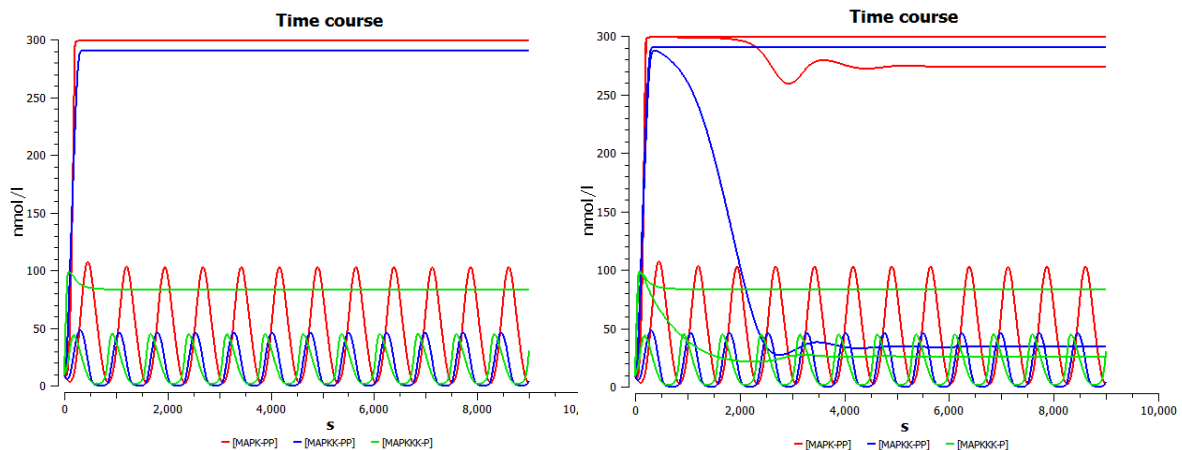
Inference:

- With smaller inhibition constants, the maximal activation achieved decreases and the frequency of oscillations increases. The system loses the oscillatory behaviour at values of Ki between 25 and 27 (you could try this by setting more intervals).

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iii.   MAPK double phosphorylation (Bistability):

> **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 two modification steps or two demodification 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. Figure 4 shows a two-step modification enzyme cycle in which both modifier and demodifier enzymes, MAPKK and MKP, follow a strictly ordered mechanism.
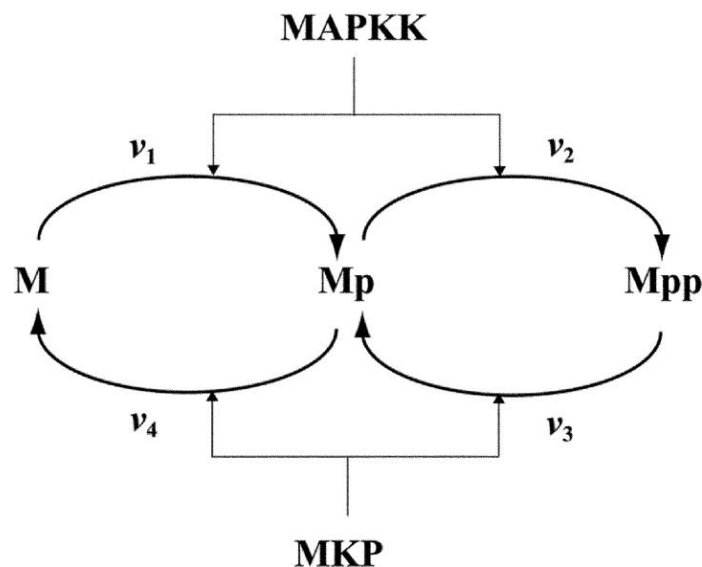

Figure 4: **Dual phosphorylation–dephosphorylation cycle of MAPK, in which both MAPKK and MKP follow distributive ordered kinetic mechanisms.** M, Mp, and Mpp stand for the unphosphorylated, monophosphorylated, and bisphosphorylated forms of MAPK. Reactions v1 and v2 are catalysed by the MAPKK, and reactions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

As illustration 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 denote 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.

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:

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 mathematical operations can be typed)
- Click on "commit"

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 "Scan" and click "Create"
- Choose the parameter, which need to be varied: Species->Initial Concentrations->[MAPKK](t=0)
- Do parameter scan: 100 intervals from 0 to 100
- Click on "Output Assistant" and choose "Scan of Concentrations, Volumes, and Global Quantity Values" to create a plot of "MAPKK" against "Mpp".
- Choose symbols for plotting. In OutputSpecification->Plots, choose Plot Type as "Symbols".
- Click "Run" to get the plot (What do you observe? You don't see the points of instable steady states)
- Add another scan
- Choose the parameter, which need to be varied: Species->Initial Concentration->[M](t=0)
- Do parameter scan: 20 intervals from 100 to 450
- Click "Run" to get the plot (This time you can see the intermediate points between two stable steady states)
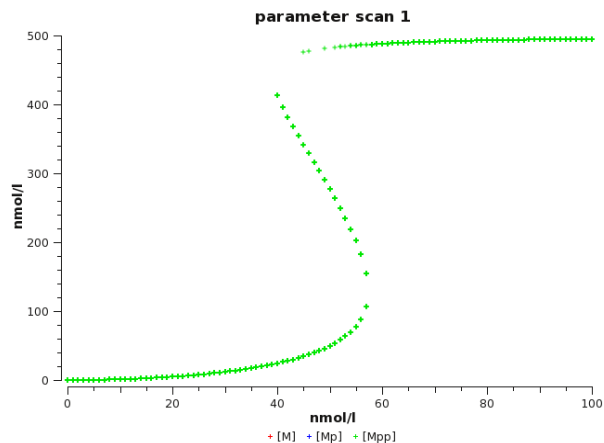
Note: Alternatively, open the file **Markevich2004_parameterscan1.cps** in COPASI (File->Open), and run parameter scan (Tasks->Parameter Scan) to get the plot (parameter scan 1).

Dependence of bistability on Km1:

- Add another scan over the global quantity Km1
- Do 1 interval from 50 to 500
- Click "Run"

Note: Alternatively, open the file **Markevich2004_parameterscan2.cps** in COPASI (File->Open), and run parameter scan (Tasks->Parameter Scan) to get the plot (parameter scan 2).
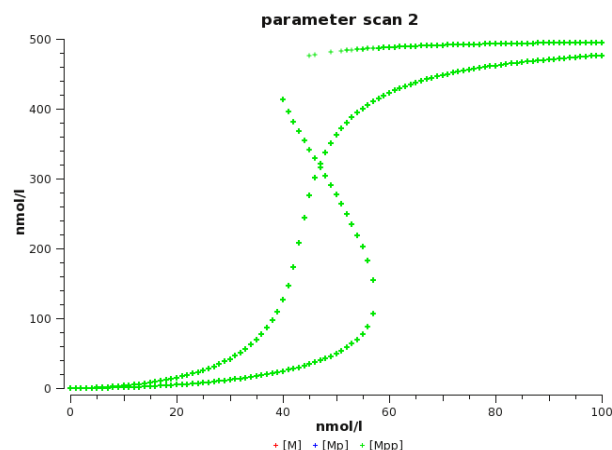
Inference:

- 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.