

Building and Simulating Models using Copasi

WTAC: In Silico Systems Biology
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Files and supporting materials are available at:
<http://www.ebi.ac.uk/biomodels/courses/20130627/>

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:

➤ Ortega F, Garcés JL, Mas F, Kholodenko BN, Cascante M. Bistability from double phosphorylation in signal transduction. Kinetic and structural requirements. FEBS J 2006 Sep;273(17):3915-3926.
(<http://www.ebi.ac.uk/biomodels-main/BIOMD0000000258>).

MAPK cascade:

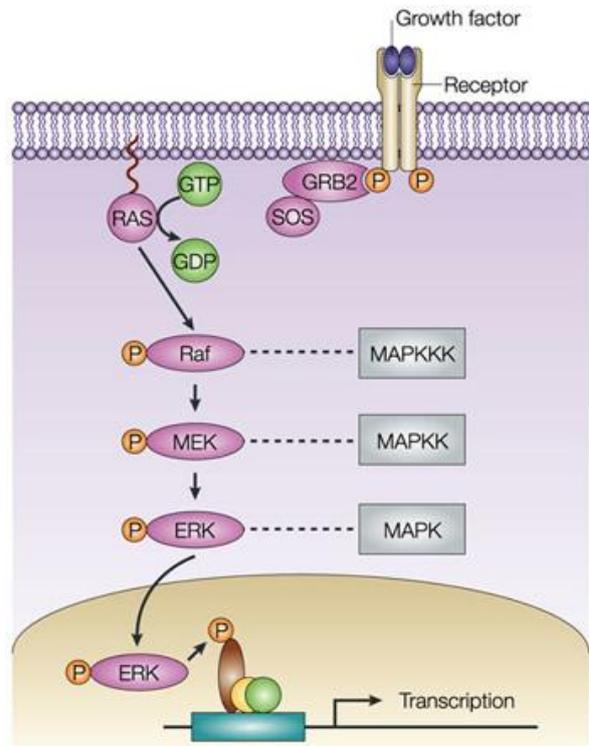
Mitogen activated protein kinase (MAPK) cascades are ubiquitous and highly conserved signalling modules, found in almost all eukaryotes. MAPK cascades are involved in many cellular processes such as cell proliferation, differentiation, movement, survival etc.

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.



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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. 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.

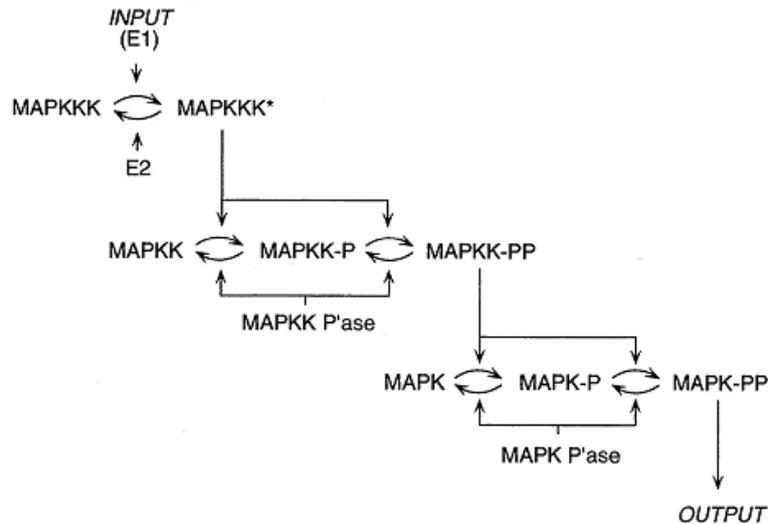
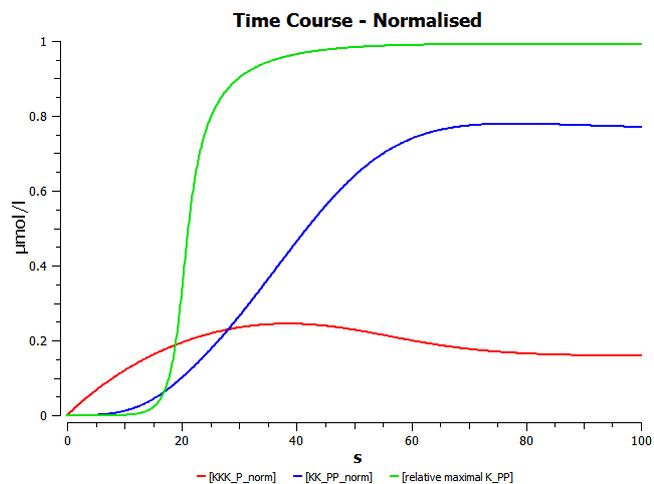


Figure 2: Schematic view of MAPK cascade.

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

- Import **BIOMD0000000009.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).
- Plot the normalized active form of MAPK (relative maximal K_PP), MAPKK (KK_PP_norm) and MAPKKK (KKK_P_norm) as a function of time. To create the plot, click on Output Assistant and choose "Concentrations, Volumes, and Global Quantity Values" in the options displayed and click Ok.
- Click on Run to get the plot.



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

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

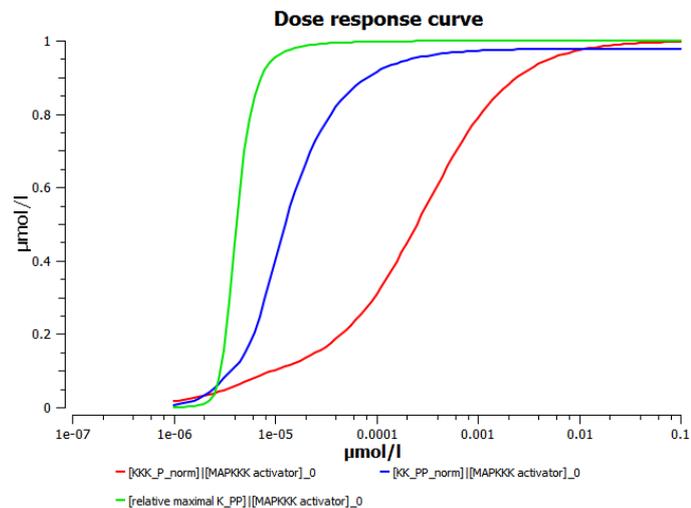
Dose response studies are carried out to understand the effect of variable amount of dose (eg. to determine the “safe” and “hazardous” levels and dosages of drugs) on the dynamics of the systems. Here, we can see the dose/stimulus response curves for three components of the MAPK cascade, the input stimulus 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.

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).
- time-course plot that was created in the previous exercise can now be deactivated.

Sensitivity to signal (dose-response curve to observe the ultrasensitivity):

- click on Tasks->Parameter Scan
- New Scan Item: Scan
- click Create
- in the Scan (blue box), under Object choose: Species->Initial Concentrations-> [MAPKKK_activator](t=0)
- set Intervals: 100; min:1e-6; max: 0.1
- check “logarithmic”
- in the Task (green box), choose “Steady State” to plot only the resulting concentration at time-> ∞ .
- Click on Run to get the dose-response curve (observe what happens??).
- In addition to the stable steady states several instable 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 **huang_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.

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, whereas 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.

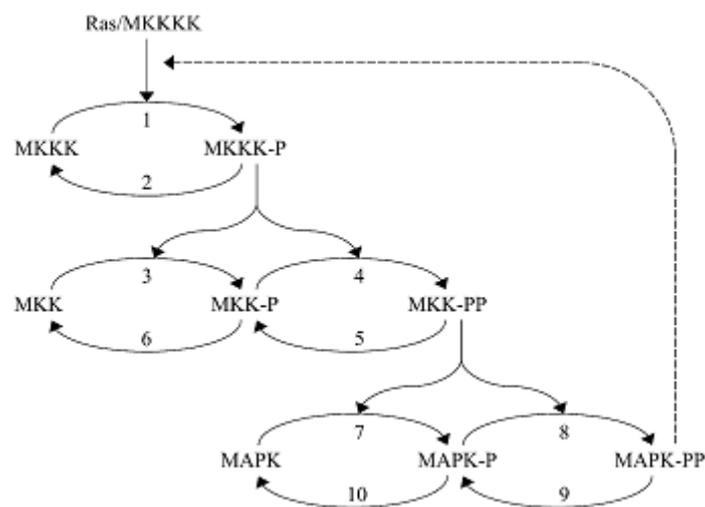


Figure 3: Kinetic scheme of MAPK cascade with negative feedback loop.

a. 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.
 - 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):
 - Function name : MM with enzyme
 - Formula : $k_{cat} \cdot E \cdot S / (K_m + S)$
 - 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:

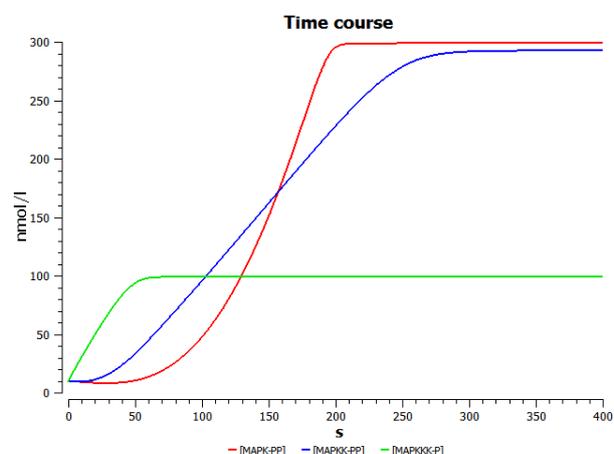
Species Name	Initial Concentration
MAPKKK	90
MAPKKK-P	10
MAPKK	280
MAPKK-P	10
MAPKK-PP	10
MAPK	280
MAPK-P	10
MAPK-PP	10

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

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

- Open **kholodenko.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 **kholodenko_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.

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

Feedback loops: Complex biological processes are regulated by feedback loops. In a sequence of event, if 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 as **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 **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, Ki, K1 and n (see below).

MAPKKK activation:

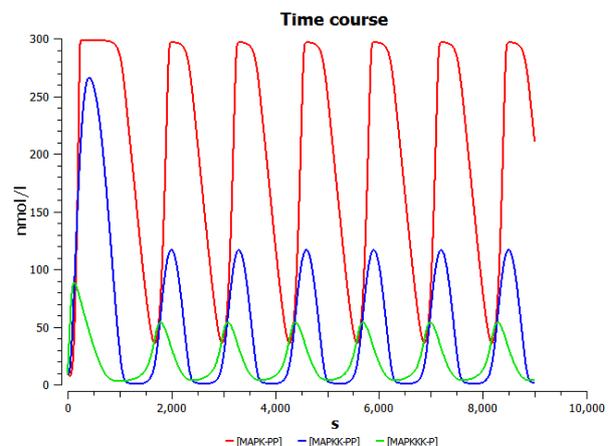
$$MAPKKK \xrightarrow{MAPK-PP} MAPKKK - P;$$

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

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).

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



Inference:

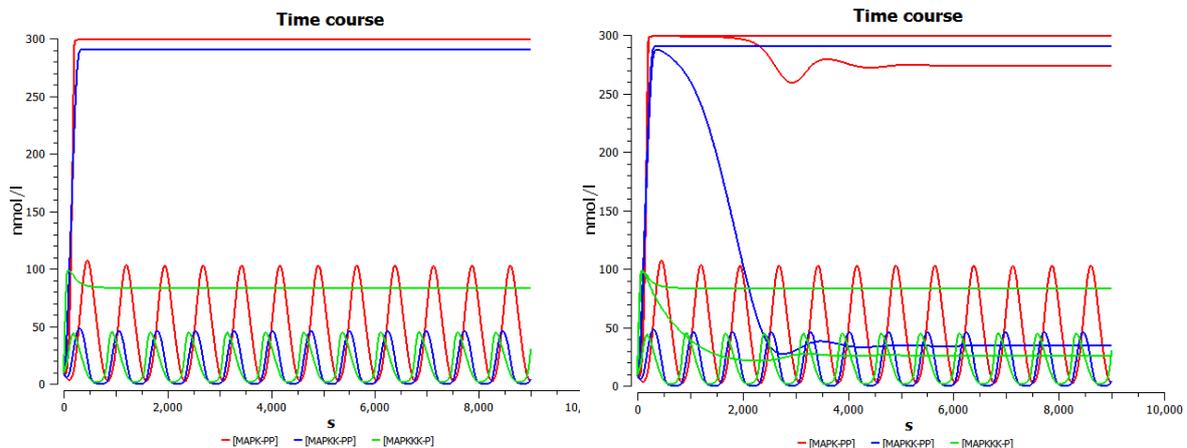
- Through the inclusion of a simple negative feedback, the system can exhibit oscillatory behaviour under constant stimulation. i.e. The combination of ultrasensitivity and negative feedback brings sustained biochemical oscillations.

d. 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 – 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).
- select Tasks->Parameter Scan
- 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 **kholodenko_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 K_i between 25 and 27 (you could try this by setting more intervals).

3. Bistability:

- Ortega F, Garcés JL, Mas F, Kholodenko BN, Cascante M. **Bistability from double phosphorylation in signal transduction. Kinetic and structural requirements. FEBS J 2006 Sep;273(17):3915-26.** (<http://www.ebi.ac.uk/biomodels-main/BIOMD000000258>).

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, e1 and e2, follow a strictly ordered mechanism. As illustrated in the kinetic diagram, a protein W has three different forms W_α (unphosphorylated), W_β (single phosphorylated) and W_γ (double phosphorylated). The four arrows indicate the interconversion between them. Steps 1 and 3 are catalysed by the same enzyme (e1), and steps 2 and 4 are catalysed by another enzyme (e2). This model can be considered as the submodel of the previous models, where W_α (unphosphorylated), W_β (single phosphorylated) and W_γ (double phosphorylated) denotes MAPK, MAPK_P and MAPK_PP, respectively.

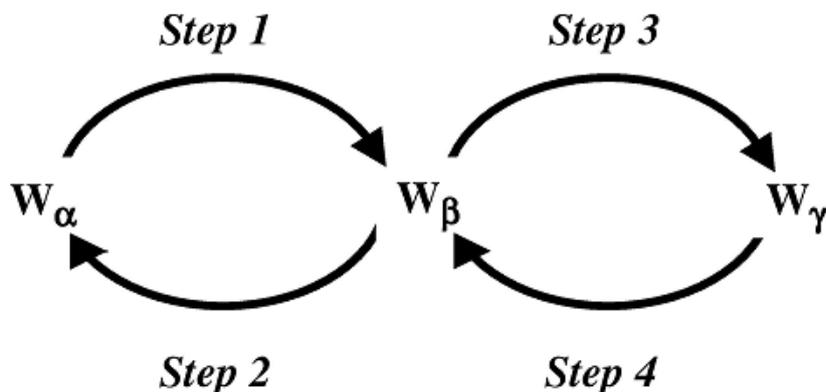


Figure 4: Kinetic diagram, in which a protein W has three different forms W_α (unphosphorylated), W_β (single-phosphorylated) and W_γ (double-phosphorylated). The four arrows show the interconversion between the different forms: $W_\alpha \rightarrow W_\beta$ (step 1); $W_\beta \rightarrow W_\alpha$ (step 2); $W_\beta \rightarrow W_\gamma$ (step 3); and $W_\gamma \rightarrow W_\beta$ (step 4). Steps 1 and 3 are catalysed by the same enzyme (e1), and steps 2 and 4 are catalysed by another enzyme (e2).

a. To perform a parameter scan to observe the bistable behaviour and hysteresis (Multisite phosphorylation can cause bistability and hysteresis):

To create a plot of “alpha” (concentration of species W_α) against “Chi14” (the ratio between the maximal activities of the first and fourth steps):

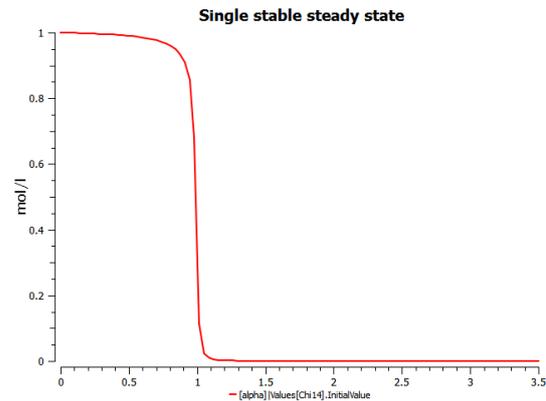
- Import **BIOMD000000258.xml** to Copasi (File->Import SBML).
- Expand the option “Output Specifications” in the left-hand side menu.
- Click on Plots
- select the tab “New” and double click on the newly generated plot.
- click “New curve”
- select Global Quantities->Initial Values-> [Chi14](t=0) for the x-axis (left)
- select Species->Transient Concentrations-> [alpha](t) for the y-axis

- (right)
- click ok (as long as the plot is ticked active, it will be updated after every calculation).

Single stable steady state:

- go to Tasks->Parameter Scan
- under "New scan item:" select "Scan" and click "create"
- choose the parameter: Global Quantities ->Initial Values-> Chi14(t=0)
- do 100 intervals from 1e-05 to 3.5
- select Task "Steady State"
- click Run to get the plot.

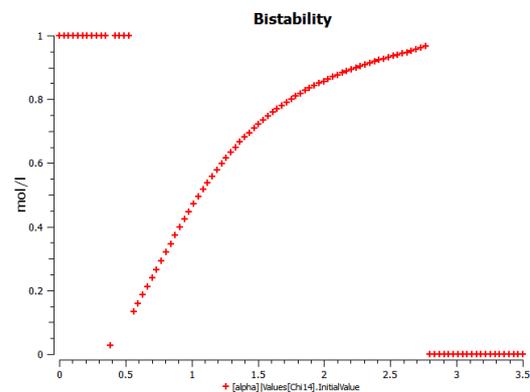
Note: Alternatively, open the file **Ortega_parameterscan_sss.cps** in Copasi and run parameter scan to get the plot.



Three steady state (2 stable steady state and 1 unstable steady state):

- expand Model->Biochemical->Global Quantities
- change the values of r24 and r31 to 6.
- go to "Plots" and change plot Type to symbol (Output Specifications->Plots).
- go to Parameter Scan.
- click Run to get the plot.

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



Inference:

- At the given parameter values, protein alpha works like a switch with a memory: whether alpha is active, does not only depend on the stimulus strength, but also on the prior state of MAPK.
- At low values of the asymmetric factor (i.e. the product of r31 and r24, where r31 and r24 are the ratios of the catalytic constants for the modification and demodification processes, respectively), there is single stable steady state (here r31=r24=1) for any value of Chi14 (the ratio between the maximal activities of the first and fourth step (i.e. ratio between the first modification and the first demodification step)).
- For a larger values of the asymmetric factor, there is a range of Chi14 values at which three steady states are possible, two of which are stable steady state and one unstable steady state (here r31=r24=6).

Do it yourself:

- Plot alpha, beta and gamma against Chi14, for different range of r31 and r24 (with r24=1, r31=1; r24=0.5, r31=1; r24=1, r31=0.5).
- Observe the effect of the ratios of the catalytic constants r31 and r24, on the variation of the steady state profiles with Chi14 (this plot is taken from figure 5 of the Ortega et al. (2006)). See if you could reproduce these plots.

