Training material for model curation:
Modelling Signalling Pathways

Files and supporting materials are available at:
http://www.ebi.ac.uk/biomodels/training/build_simulate/

Download Copasi lastest stable version 4.12 (Build 81) from:
http://www.copasi.org/tiki-index.php?page_ref_id=106#stable

Download SBMLeditor from:
http://www.ebi.ac.uk/compneur-srv/SBMLeditor.html

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Exercise:

I. Encoding model using SBMLeditor:

- 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/BIOMD0000000258).

II. Model Simulation using COPASI:

i. Bistability (double phosphorylation):

- 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/BIOMD0000000258).

ii. 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)

III. Model building and simulation using COPASI:

i. 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)

IV. Additional simulation exercise using COPASI:

i. p53/MDM2 circuit (These models have discrete events):

- Proctor CJ, Gray DA. Explaining oscillations and variability in the p53-Mdm2 system. BMC Syst Biol. 2008 Aug 18;2:75.
  p53 stabilization by ATM - http://www.ebi.ac.uk/biomodels-main/BIOMD0000000188
  p53 stabilization by ARF - http://www.ebi.ac.uk/biomodels-main/BIOMD0000000189

ii. Repressilator:

- Elowitz MB, Leibler S. A synthetic oscillatory network of transcriptional regulators. Nature 2000 Jan;403(6767):335-8. (http://www.ebi.ac.uk/biomodels-main/BIOMD0000000012)

I. Encoding model using SBMLeditor:

- 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/BIOMD0000000258).

i. Open SBMLeditor and click File-New (a new model header is created).

ii. Create a compartment "cell" with volume = 1
- right click on model:model01(model), then click Add->listOfComparments
- right click on listOfCompartments and select "Add New Comparment".
- create a compartment cell with, id = cell; name = cell; size = 1.

iii. Encoding species with initial concentrations:
- right click on model:model01(model), then click Add->listOfSpecies
- right click on listOfSpecies and select "Add New Species".
- create the following species one by one.

Species Id/Names	Initial Concentrations
Alpha	0.462
Beta	0.2
Gamma	0.338

iv. Encoding global parameters with their values:
- right click on model:model01(model), then click Add->listOfParameters.
- right click on listOfParameters and select "Add New Parameter"
- create the following parameters one by one.

Parameter Id/Name	Parameter Value
r31	1.0
r24	1.0
Chi14	1.1
Ks1	0.01
Ks2	0.01
Ks3	0.01
Ks4	0.01
Vm1	1.0
P	1.0

v. Encoding reactions with kinetic law, reactants, products and modifiers:
- right click on model:model01(model), then click Add->listOfReactions.
- right click on listOfReactions and select "Add New Reaction"
- create the following reactions one by one (include kinetic law, reactants, product and modifier).

Reaction Id/Name	Reaction	Modifier	KineticLaw (Math)
v1	alpha → beta	-	Vm1 * (alpha/Ks1)/(1+alpha/Ks1+beta/Ks3)
v2	beta → alpha	Gamma	r24 * (Vm1 / Chi14) * (beta / Ks2) / (1 + gamma / Ks4 + beta / Ks2)
v3	beta→ gamma	Alpha	r31 * Vm1 * (beta / Ks3) / (1 + alpha / Ks1 + beta / Ks3)
v4	gamma → beta	-	Vm1 / Chi14 * (gamma / Ks4) / (1 + gamma / Ks4 + beta / Ks2)

vi. save the file (say **bistability.xml**. The file has extension .xml).

II. Model Simulation using COPASI:

i. Bistability (double phosphorylation):

- **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/BIOMD0000000258**).**

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 1 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 Wα (unphosphorylated), Wβ (single phosphorylated) and Wγ (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 Wα (unphosphorylated), Wβ (single phosphorylated) and Wγ (double phosphorylated) denotes MAPK, MAPK_P and MAPK_PP, respectively.

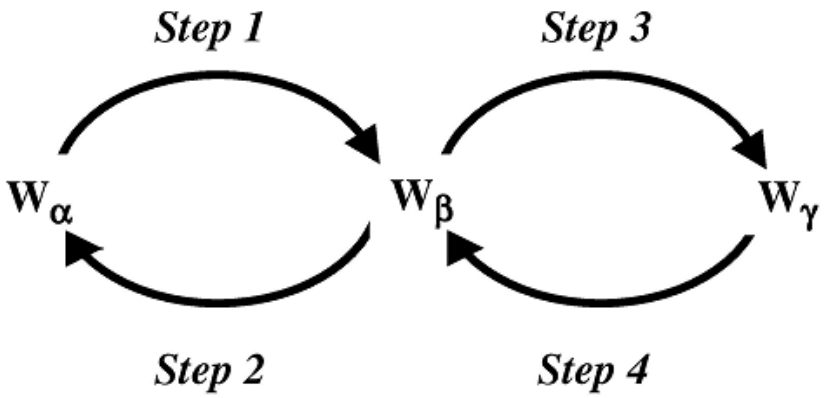


Figure 1: Kinetic diagram, in which a protein W has three different forms Wα (un-phosphorylated), Wβ (single-phosphorylated) and Wγ (double-phosphorylated). The four arrows show the interconversion between the different forms: Wα->Wβ (step 1); Wβ->Wα (step 2); Wβ->Wγ (step 3); and Wγ->Wβ (step 4). Steps 1 and 3 are catalyzed by the same enzyme (e1), and steps 2 and 4 are catalysed by another enzyme (e2).

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 Wα) against "Chi14" (the ratio between the maximal activities of the first and fourth steps):

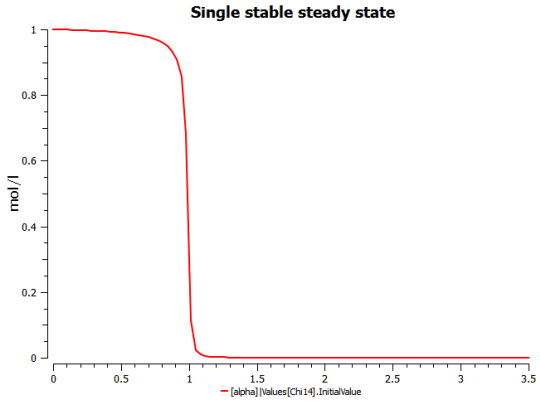
- Import the model encoded in the previous section (**bistability.xml**) to Copasi (File->Import SBML).
- Expand the option "Output Specifications" in the left-hand side menu.
- Click on Plots
- select the tab "New" and double click on the newly generated plot.
- click "New curve"
- select Global Quantities->Initial 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).

Note: Alternatively, import **BIOMD0000000258.xml** in Copasi and do the above.

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.

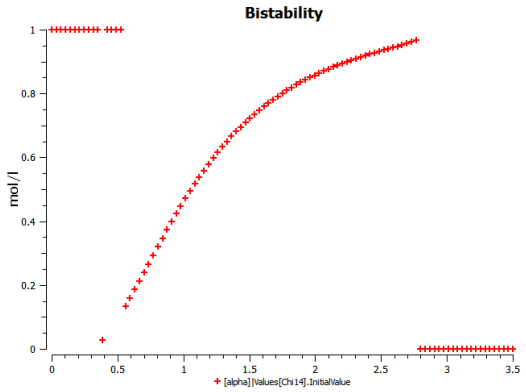
Note: Alternatively, open the file **Ortega_parameterscan_sss.cps** in Copasi and run parameter scan to get the plot.



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.

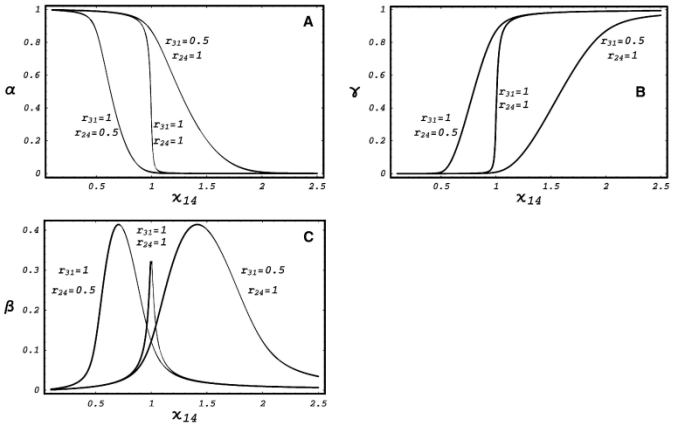
Note: Alternatively, open the file **Ortega_parameterscan_bistable.cps** in Copasi and run parameter scan to get the plot.



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:



V. 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).

VI. 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.

ii. MAPK cascade (Ultrasensitivity):

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 2).

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.

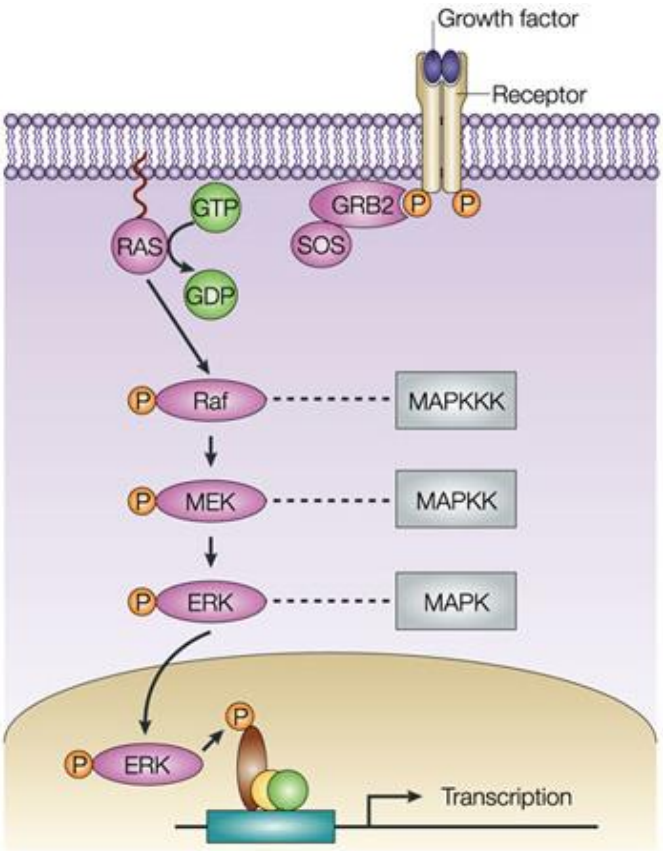


Figure 2:MAPK cascade. Figure taken from http://www.nature.com/nrm/journal/v5/n6/box/nrm1400_BX1.html

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

- **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 3). 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 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.

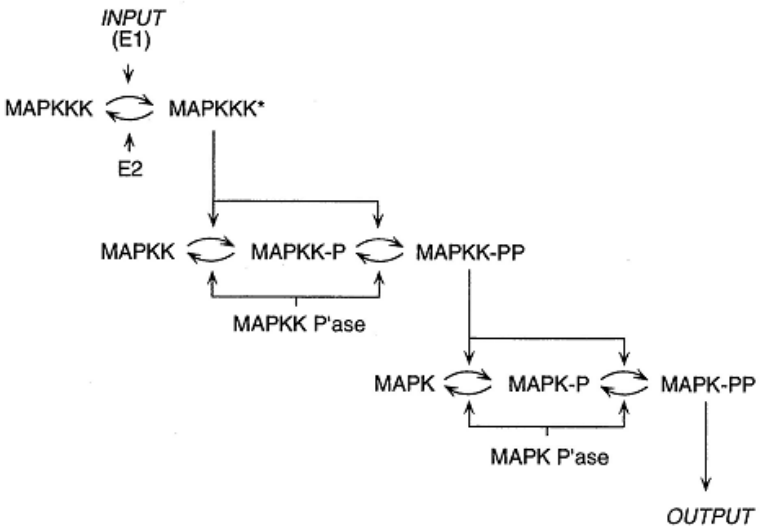
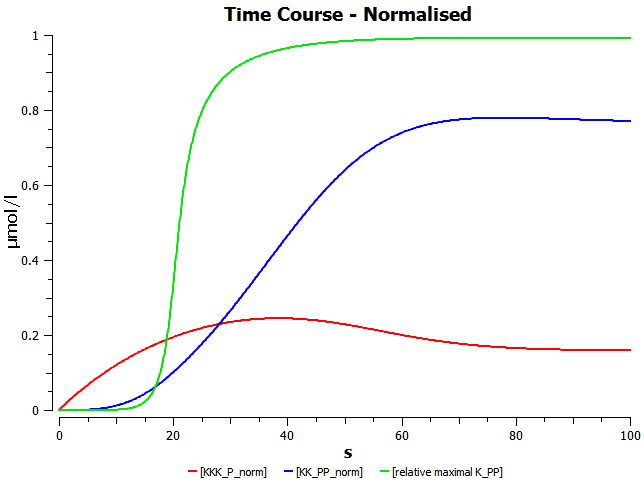


Figure 3: Schematic view of MAPK cascade.

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 Assisant 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.

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 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:

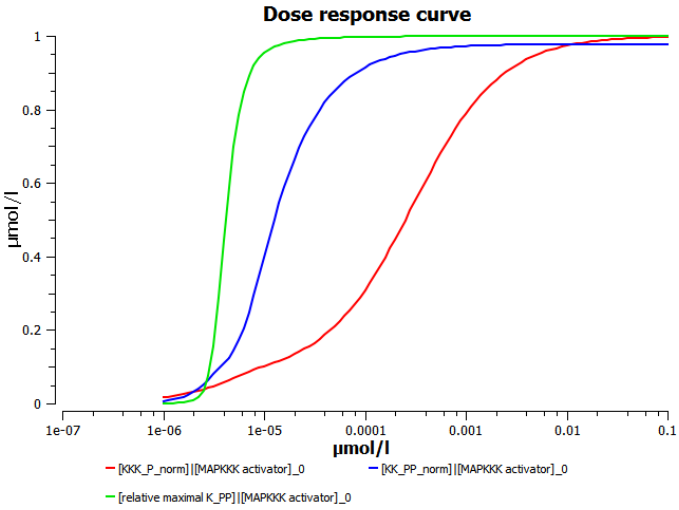
- expand the option "Output Specifications" in the left-hand side menu.
- click on Plots
- select the tab "New" and double click on the newly generated plot.
- click "New curve"
- select Species->Initial Concentrations-> [MAPKKK_activator](t=0) for the x-axis (left)
- select Species->Transient Concentrations-> [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 see ultra sensitivity):

- 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:
- 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.

III. Model building and simulation using COPASI:

i. 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 4). Indeed, while tyrosine phosphorlated 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 (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 Ferrel model where the reactions are modelled using mass-action kinetics.

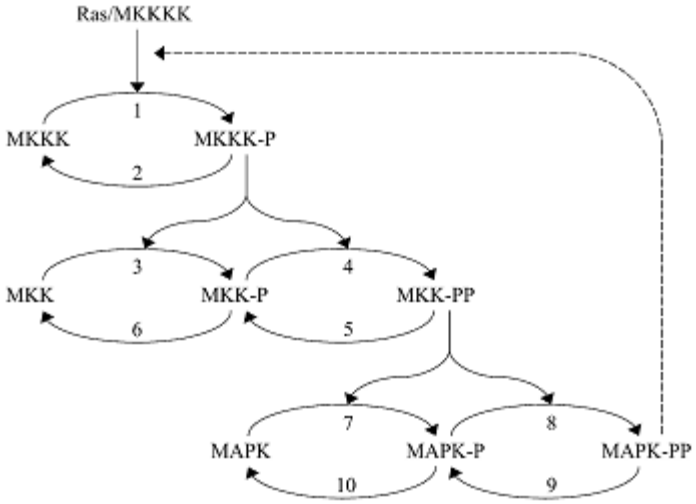


Figure 4: Kinetic scheme of MAPK cascade with negative feedback loop.

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):

  Function name : 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:

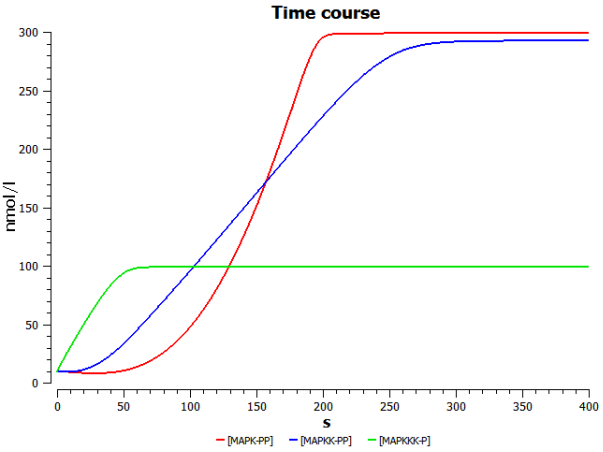
Species Name	Initial Concentration
MAPKKK	90
MAPKKK-P	10
MAPKK	280
MAPKK-P	10
MAPKK-PP	10
MAPK	280
MAPK-P	10
MAPK-PP	10

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

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

- Open **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.

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 **kholodenko.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:

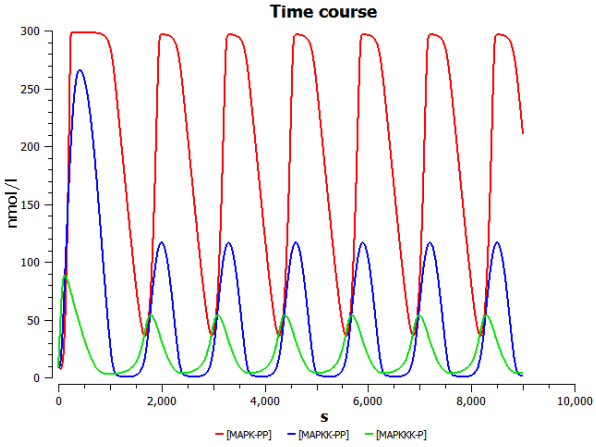
$$MAPKKK \xrightarrow{MAPK-PP} MAPKKK-P;$$

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

Note: If you have not added the new function definition "HMM with inhibition" as above, in **kholodenko.cps**, then as an alternative open **kholodenko_nf.cps** or import **BIOMD0000000010.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:

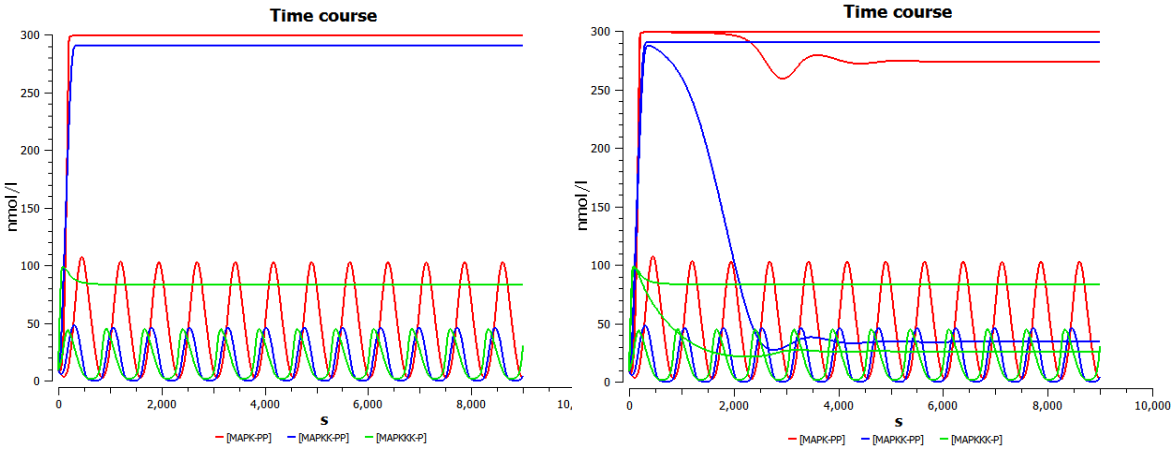
- Through the inclusion of a simple negative feedback, the system can exhibit oscillatory behaviour under constant stimulation. i.e. The combination of ultrasensitivity and negative feedback brings sustained biochemical oscillations.

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 pervious 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 Ki between 25 and 27 (you could try this by setting more intervals).

IV. Additional simulation exercise using COPASI:

i. p53/MDM2 circuit:

- **Proctor CJ, Gray DA. Explaining oscillations and variability in the p53-Mdm2 system. BMC Syst Biol. 2008 Aug 18;2:75.**

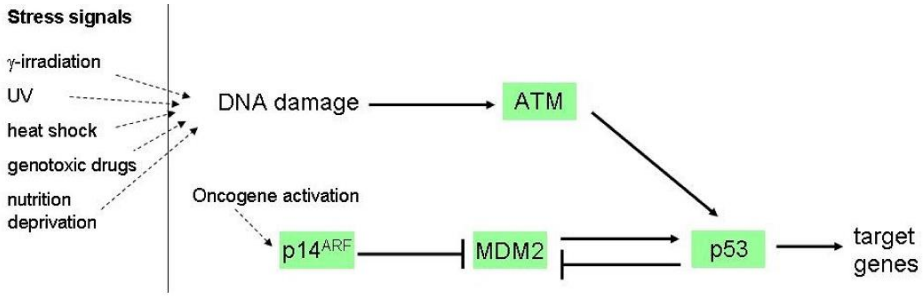


Figure 6: Network diagram of p53 signaling pathway.

The p53 protein is a sequence-specific DNA binding transcription factor which is encoded by TP53 gene (in human) located on the short arm of chromosome 17. Three major functions of p53: growth arrest, DNA repair and apoptosis.
In normal cell, p53 concentration is regulated by Mdm2 (negatively regulated) since, excess of p53 may accelerate the aging process by excessive apoptosis. In stressed cell: Its important function in response to DNA damage is to induce cell growth arrest, to allow DNA repair to take place, by which it prevents tumour growth (p53 also called as tumour suppressor). If the DNA repair fails, it initiates Apoptosis.
DNA damage sensed by kinases like ATM and nucleolar protein ARF inhibits Mdm2 binding to p53 resulting in p53 stabilization and hence it initiates growth arrest and activates transcription of protein involved in DNA repair (Figure 6).

Here, we will see two models:

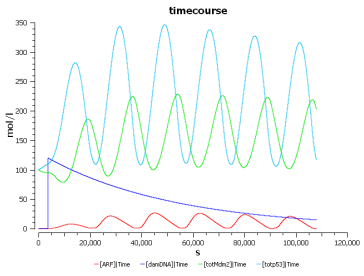
p53 stabilization by ARF - http://www.ebi.ac.uk/biomodels-main/BIOMD0000000189
p53 stabilization by ATM - http://www.ebi.ac.uk/biomodels-main/BIOMD0000000188

These models contain events.
Events: Events are defined as an instantaneous, discontinuous change in a variable, for example, triggering certain process at a particular time and stop it at a particular time.
In the above two models, DNAdamage is initiated by irradiation (IR=25dGy for 1minute at time t = 1hour).

p53 stabilization by ARF:

Import **BIOMD0000000189.xml** to COPASI and do the following:

- Run a time-course simulation for 30 hours (108000 seconds), using deterministic algorithm.(Tasks->Time Course)
- Plot the total p53 (totp53), total Mdm2 (totMdm2), amount of damaged DNA (damDNA) and ARF as a function of time.



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

Inference:

- Observe how the amplitude of the oscillations of total p53 and

total Mdm2 vary, when the DNA damage is triggered and the gradual disappearance of the oscillations when the DNA is repaired.

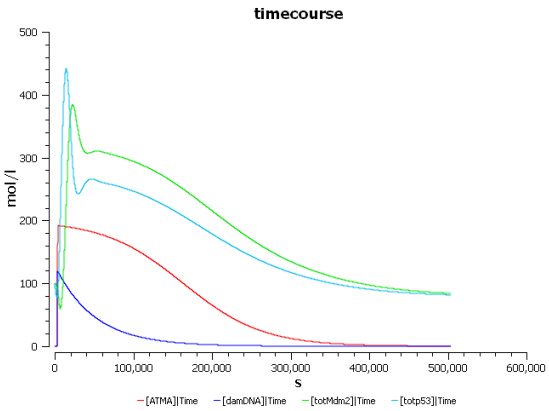
- Observe the increase in ARF concentration during the DNA is being repaired and its reduction as the DNA is repaired. (DNA damage is sensed by the nucleolar protein ARF, which then binds to Mdm2 and sequesters it, to up-regulate the transcriptional activity of p53 in order to repair the DNA.)

p53 stabilization by ATM:

Import **BIOMD0000000188.xml** to COPASI and do the following:

- Run a time-course simulation for 30 hours (108000 seconds), using deterministic algorithm.(Tasks->Time Course)
- Plot the total p53 (totp53), total Mdm2 (totMdm2), amount of damaged DNA (damDNA) and active form of ATM (ATMA) as a function of time.

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



Inference:

- Observe the plot obtained for total p53 (totp53) and total Mdm2 (totMdm2) when the DNA damage is triggered and the reach of steady state as the DNA is repaired.
- Observe the increase in the active form of ATM (ATMA) concentration during the DNA is being repaired and its reduction as the DNA is repaired. When it reaches “zero” p53 and Mdm2 reaches its steady state. (DNA damage is sensed by 'checkpoints' in the cell cycle, and causes protein kinases such as ATM to phosphorylate p53 at the N-terminal transcriptional activation domain which has a large number of phosphorylation sites (sites that are close to or within the MDM2-binding site of p53). Phosphorylation of the N-terminal end of p53 by kinases disrupts Mdm2-binding. In addition, Mdm2 also gets phosphorylated which also enhances the degradation of Mdm2. Thus, p53 gets stabilized and its transcriptional activity gets up-regulated in order to repair the DNA.)

ii. Repressilator:

- Elowitz MB, Leibler S. A synthetic oscillatory network of transcriptional regulators. Nature 2000 Jan;403(6767):335-8. (http://www.ebi.ac.uk/biomodels-main/BIOMD0000000012)

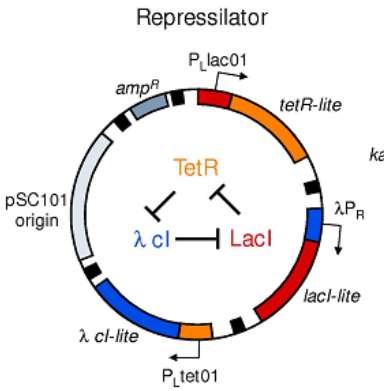


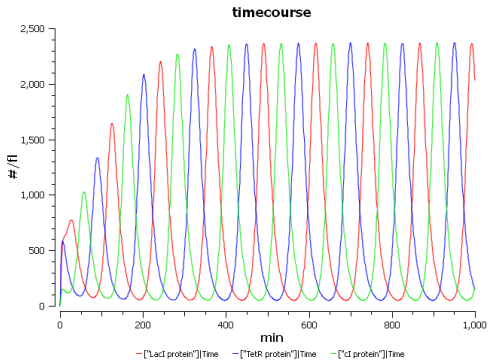
Figure 7: The repressilator network. The repressilator is a cycle negative feedback loop composed of three repressor genes and their corresponding promoters, as shown schematically in the centre of the left-hand plasmid.

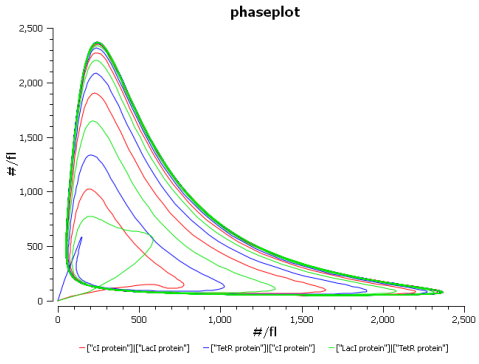
Elowitz and Leibler 2000, designed and constructed a synthetic network which involves three transcriptional repressor system in e.coli to show the oscillatory behaviour. The model contains loop of 3 transcriptional repressors each controlling the next (Figure 7) – negative feedback. Here, we could see the oscillatory behaviour of the repressor proteins "LacI", "cI" and "TetR". Also, can see what happens when altering some of the key parameters.

Negative feedback loop and oscillation:

Import **BIOMD0000000012.xml** to COPASI and do the following:

- run a time-course simulation for 1000 minutes using a deterministic algorithm. (Tasks->Time Course)
- Create a plot of lacI, cI and tetR against time.
- Observe the oscillations (plot in the left side).
- Phase plot: Create a plot of "lacI" against "cI", "cI" against "tetR" and "tetR" against "lacI".
- Observe the temporal evolution of the repressor proteins lacI, tetR and cI in phase-plane (plot in the right side).





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

Inference:

- The model encodes the loop of the three transcriptional repressors, each controlling the next – negative feedback.
- Here, the oscillatory behaviour and the phase-plane plot of the repressor proteins “LacI”, “cI” and “TetR” are illustrated.

Do it yourself:

- What happens if you put all mRNA and protein concentrations to the same number?
- Observe the system by changing key parameters (eg. Change n, the Hill coefficient to 1.8, 1.75, 1.5 and 1 or change the protein half life to 25 and 50 minutes and the mRNA half life to 1 and 0.5 minutes. You might need to increase the simulation time and print steps).