Hands on Tutorial:
Modeling Signaling Pathways

The 11th International Conference on Systems Biology (ICSB)
Edinburgh International Conference Centre
Edinburgh
10th October 2010

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Files and supporting materials are available at:
http://www.ebi.ac.uk/~viji/BIOMD-tutorial/ICSB_10Oct2010/

Download Copasi last stable version 4.6 (Build 32 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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Exercises:

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:

1. 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).

2. MAPK cascade:

> 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)

> Kholodenko BN. Negative feedback and ultrasensity 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)

Additional Exercises:

3. 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

4. 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)

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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).

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II. Model Simulation using COPASI:

1. 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**).**
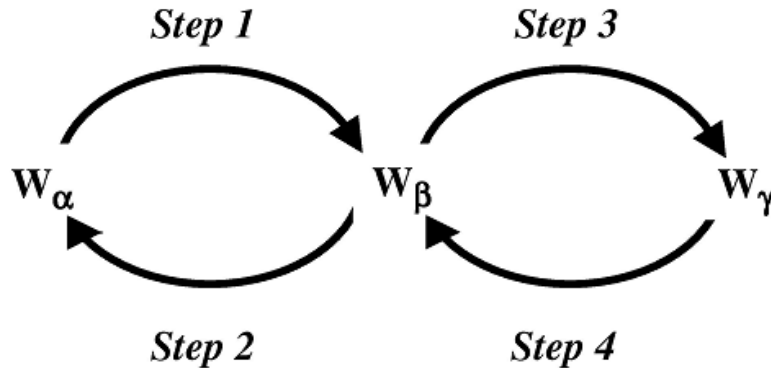

Figure 1: Kinetic diagram, in which a protein W has three different forms Wα (un-phosphorylated), Wβ (single-phosphorylated) and Wγ (double-phosphorylated). The four arrows show the interconversion between the different forms: Wα → Wβ (step 1); Wβ → Wα (step 2); Wβ → Wγ (step 3); and Wγ → Wβ (step 4).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

About the model:

    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).

Simulation:

    Import the SBML file **bistability.xml (**generated using SBMLeditor) or import **BIOMD0000000258.xml** to Copasi (File->Import SBML) and do the following:

    To create a plot of "alpha" against "Chi14":

* 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-> [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).

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Running a parameter scan to see the bistable behaviour and the hysteresis:
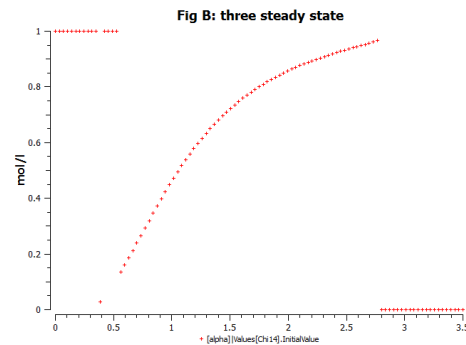
Single stable steady state:

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

Fig A: single stable steady state
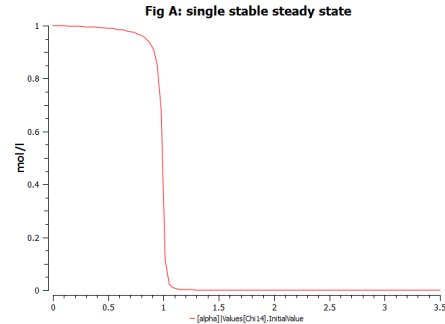
Three steady state (2 stable steady state and 1 unstable steady state):

- 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->stead state).
- go to Parameter Scan.
- click Run to get the plot.

Fig B: three steady state

Observations:

- 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 (Fig A; 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 (Fig B; 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 (Figure 2: this plot is taken from figure 5 of the Ortega 2006 et. al.). See if you could reproduce these plots.
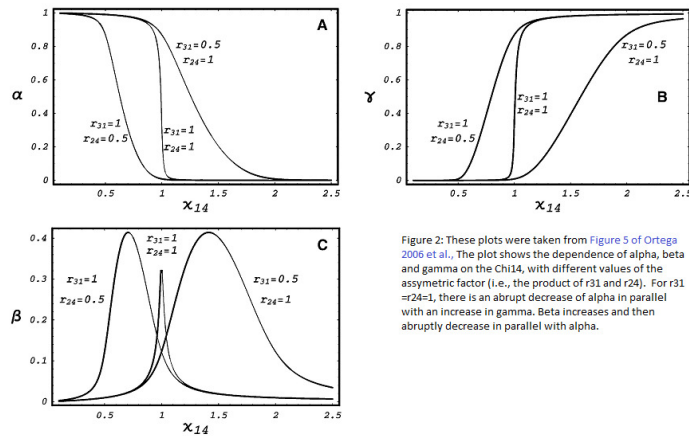

Figure 2: These plots were taken from Figure 5 of Ortega 2006 et al., The plot shows the dependence of alpha, beta and gamma on the Chi14, with different values of the asymmetric factor (i.e., the product of r31 and r24). For r31 =r24=1, there is an abrupt decrease of alpha in parallel with an increase in gamma. Beta increases and then abruptly decrease in parallel with alpha.

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2. MAPK cascade:

Mitogen activated protein kinase (MAPK) cascades are ubiquitous signaling modules that couple receptor mediated events at the cell surface to cytoplasmic and nuclear effectors.

In mammalian cells, one well characterized Signal transduction pathways – links the activated receptor tyrosine kinases (RTKs) to the MAPK cascades (Figure 3).

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 activates the membrane bound GTPase RAS.

Activated RAS triggers the activation of MAPKKK (Raf).

MAPK signaling cascades – signal-relay mechanism involves sequential phosphorylation of three kinases (Ser/Thr protein kinase).

Involve in many cellular processes such as cell proliferation, differentiation, movement, survival etc.). Widely conserved among eukaryotes.

The cascade arrangement has important consequences for the dynamics (switch like or all-or-none and oscillatory activation pattern) of MAPK signaling.
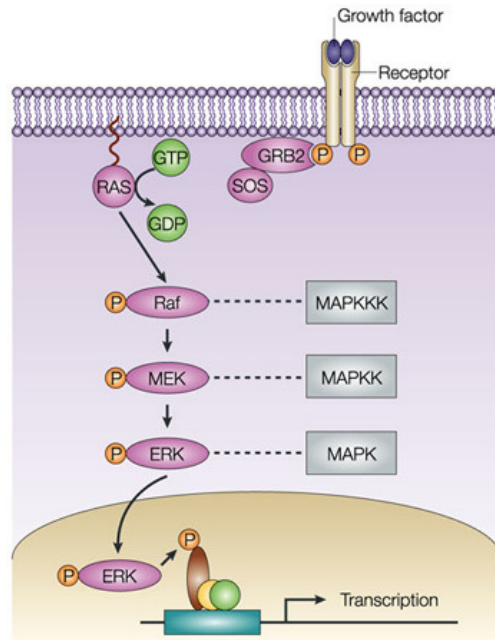
Figure 3: MAPK cascade. Figure taken from
http://www.nature.com/nrm/journal/v5/n6/box/nrm1400
_BX1.html

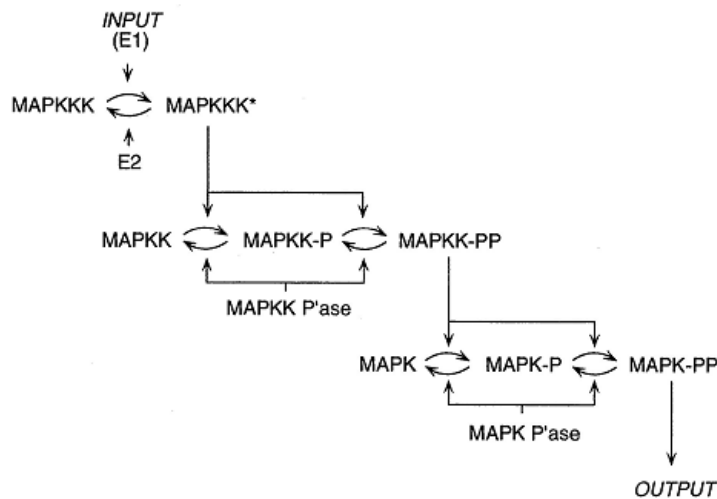> **Huang CY, Ferrell JE Jr. Ultrasensitivity in the mitogen-activated protein kinase cascade. Proc Natl Acad Sci U S A. 1996 Sep 17;93(19):10078-83. (**http://www.ebi.ac.uk/biomodels-main/BIOMD0000000009**)**

Figure 4: Schematic view of MAPK cascade.

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About the model:

This model demonstrates that the cascade arrangement has unexpected consequences for the dynamics of the MAPK signaling (Figure 4). 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 the experimental result. The experimental result in Xenopus *laevis* oocytes reproduced the predicted result.

Simulation:

Import **BIOMD0000000009.xml** to COPASI and do the following:

- Run a time-course simulation of 150 seconds, using deterministic algorithm.(Tasks->Time Course)
- Plot the normalized active form of MAPK (K_PP_norm), MAPKK (KK_PP_norm) and MAPKKK (KKK_P_norm) as a function of time.

  Note: Alternatively, open the file, **huang_timecourse.cps** in COPASI and run time-course to get the plot.
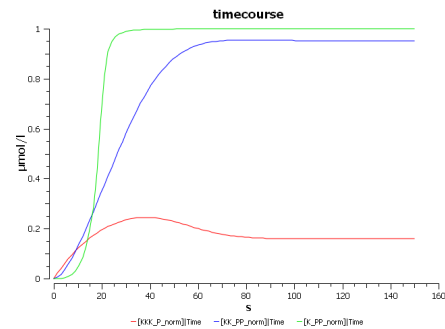
Sensitivity to signal (observe the variations in steepness of the curves due to ultra sensitivity):

- Perform parameter 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 (select logarithmic scale). (Tasks->Parameter Scan)
- Choose subtask "Steady State" to plot only the resulting concentration at time->∞
- Plot the normalized active form of MAPK (K_PP_norm), MAPKK (KK_PP_norm) and MAPKKK (KKK_P_norm) at steady state as a function of the initial activity of MAPKKK_activator (E1). Choose Axes scales as log X-axis.
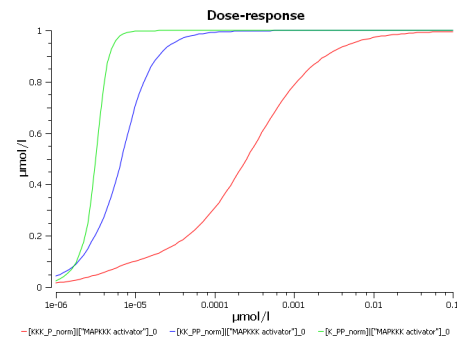
  Note: Alternatively, open the file **huang_parameterscan.cps** in COPASI and run parameter scan to get the plot.

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 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.

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➢ **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**)**
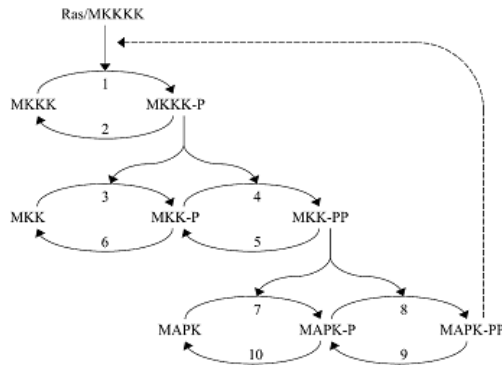

Figure 5: Kinetic scheme of MAPK cascade with negative feedback loop.

About the model:

Inhibitory phosphorylation of SOS by p42/p44 MAPK (ERK) provides a mechanism for switching off Ras signaling. This inhibition creates a negative-feedback in the MAPK cascade (Figure 5). Indeed, whereas tyrosine phosphorlated Raf brings ERK activation, ERK mediated inhibition of Raf stimulation by SOS decrease ERK phosphorylation. Combination of negative feedback and ultrasensitivity brings in sustained biochemical oscillation.

Simulation:

Open **kholodenko.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, MAPKK and MAPKKK in function of time.
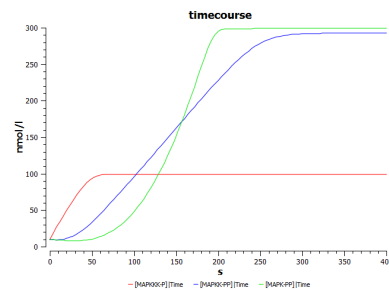
  Note: Alternatively, open the file **kholodenko_timecourse.cps** in Copasi and run time-course to get the plot.

Sensitivity to signal:

Perform the following parameter scan to simulate varying signal strength by opening kholodenko.cps:
- Vary V (Reactions-> Reaction Parameters-> MAPKKK activation->V) 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, MAPKK and MAPKKK at steady state as a function of the initial activity of Ras (V1).
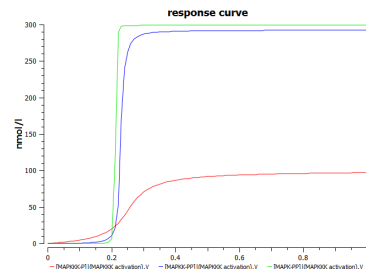
  Note: Alternatively, open the file **kholodenko_parameterscan.cps** in COPASI and run parameter scan to get the plot.

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<u>Negative feedback:</u>

- Replace the kinetic describing the phosphorylation of MAPKKK (i.e. MAPKKK activation) by the following: (add a new function in kholodenko.cps).
  - Function name : function_4_MAPKKK activation
  - 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-Menton (irreversible)" of MAPKKK activation by the new function "function_4_MAPKKK activation" and change the parameter values for V1, Ki, K1 and n (see below).

  <u>MAPKKK activation</u>:

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

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

  Note: If you have not added the new function "function_4_MAPKKK activation" as above in kholodenko.cps, then as an alternative <u>open</u> **kholodenko_nf.cps** or <u>import</u> **BIOMD0000000010.xml** (which has negative feedback already added) and do the following.

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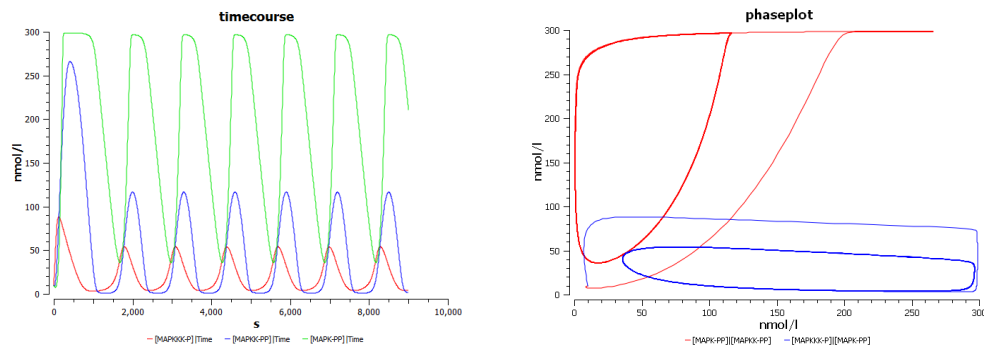
- Plot the active form of MAPKKK as a function of the active form of MAPK and the active form of MAPK as a function of the active form of MAPKK (plot in the right side).

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

<u>Dependence on inhibition:</u>

  Perform the following parameter scan:

- Vary K_i from 1 to 51 in a linear fashion.
- This simulates a decrease of the inhibitory feedback (K_i indicates the concentration of the inhibitor needed fro half-maximal inhibition).
- Plot time-course of the resulting active forms of all the three

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forms of MAP Kinases and look at the change in the oscillation patterns (plot in the left side).

- Also plot the active form of MAPKKK as a function of the active form of MAPK and active form of MAPK as a function of the active form of MAPKK (plot in the right side).

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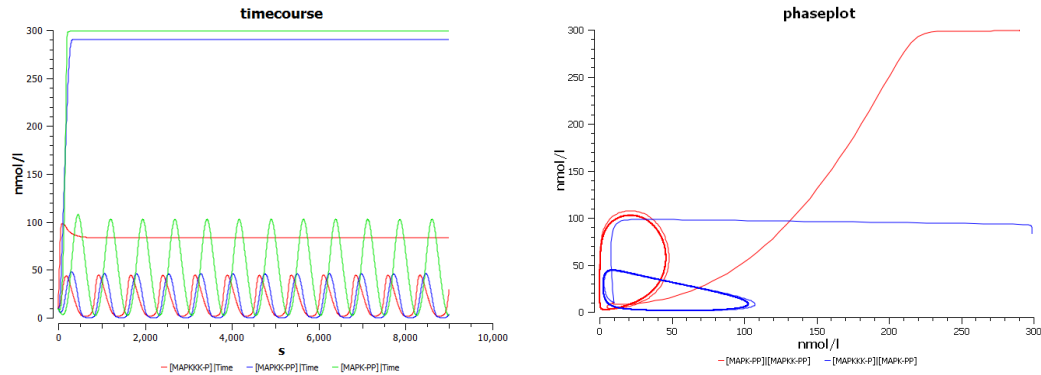
Observations:

- This model shows similar temporal activation profiles and increase of sensitivity to the one 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 achieved maximal activation decreases, and the frequency of oscillations increases. The system looses the oscillatory behaviour at values of Ki between 25 and 27.

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Additional Exercises (do the following if you have completed the above):

3. p53/MDM2 circuit:

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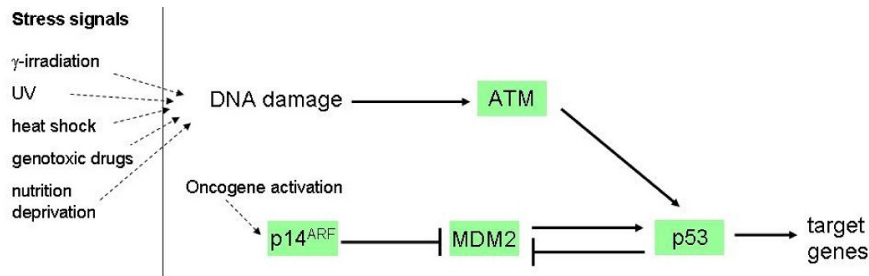

Figure 6: Network diagram of p53 signaling pathway.

About the model:

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:

Simulation:

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.

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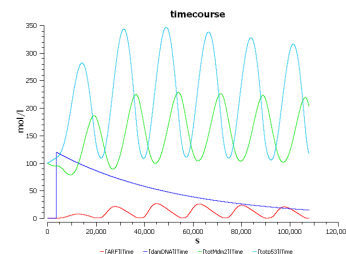
Observations:

- 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:

Simulation:

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.
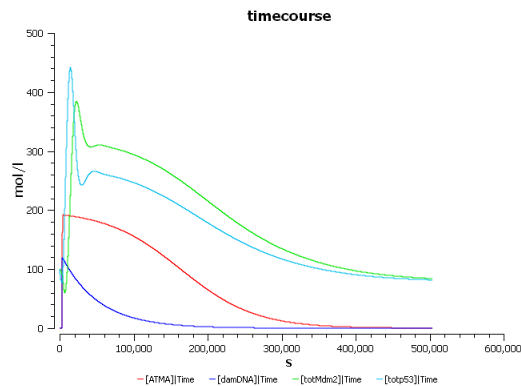
Observations:

- 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.)

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4. Repressilator:

i. 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)
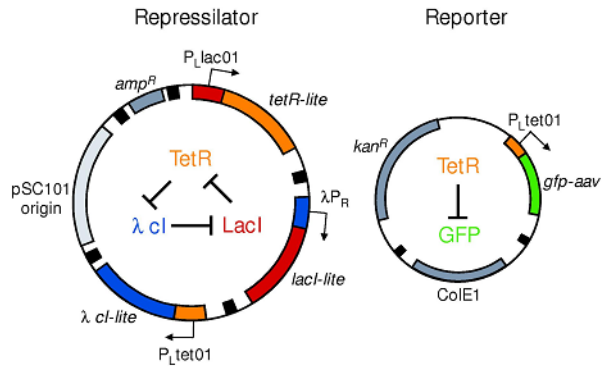

Figure 7: The repressilator network. The repressilator is a cycle negative feedback loop composed of three repressor genes and their corresponding promoters, as shown schematically in the centre of the left-hand plasmid.
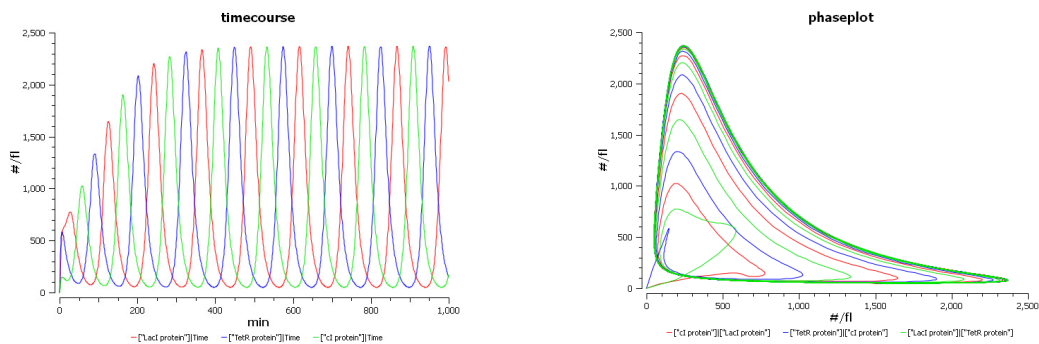
About the model:

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", "cl" and "TetR". Also, can see what happens when altering some of the key parameters.

Simulation:

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, cl and tetR against time.
- Observe the oscillations (plot in the left side).
- Phase plot: Create a plot of "lacI" against "cl", "cl" against "tetR" and "tetR" against "lacI".
- Observe the temporal evolution of the repressor proteins lacI, tetR and cl 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.

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Observations:

- 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).

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