Hands on Tutorial:
Modeling Signaling Pathways
using COPASI

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Files and supporting materials are available at:
http://www.ebi.ac.uk/~viji/13April2010

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Exercises:

I. Main:

1. MAPK cascade:

i. 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/BIOMD0000000009)

ii. 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/BIOMD0000000010)

II. Additional:

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

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

3. 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/BIOMD0000000012)

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1. MAPK cascade:

i. **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/BIOMD0000000009**)**
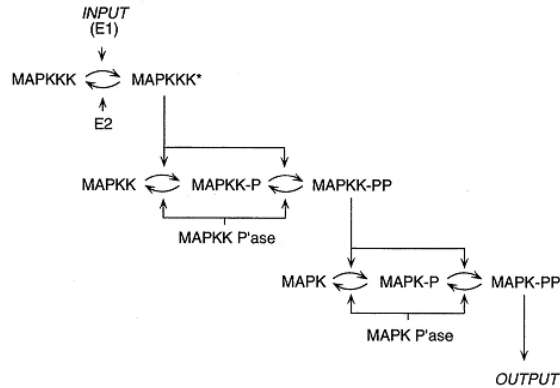

Figure 1: Schematic view of the MAPK cascade.

Simulation:

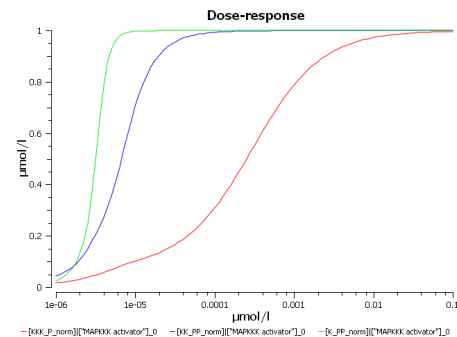Import **BIOMD0000000009.xml** to COPASI and do the following:

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

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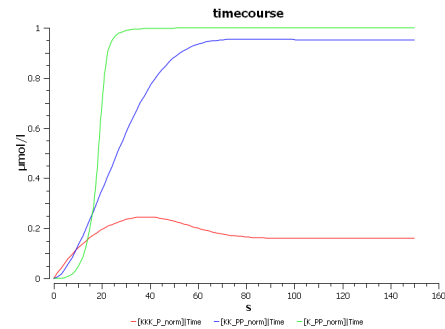
Sensitivity to signal (observe the variations in steepness of the curves due to ultra sensitivity):

- 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 MAP kinase (K_PP_norm), MAP kinase kinase (KK_PP_norm) and MAP kinase kinase kinase (KKK_P_norm) at steady state as a function of the initial activity of MAPKKK_activator (E1). Choose Axes scales as log X-axis.

File: **huang_parameterscan.cps** (open in COPASI and run parameter scan to get the plot)

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ii. 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/BIOMD0000000010**)**
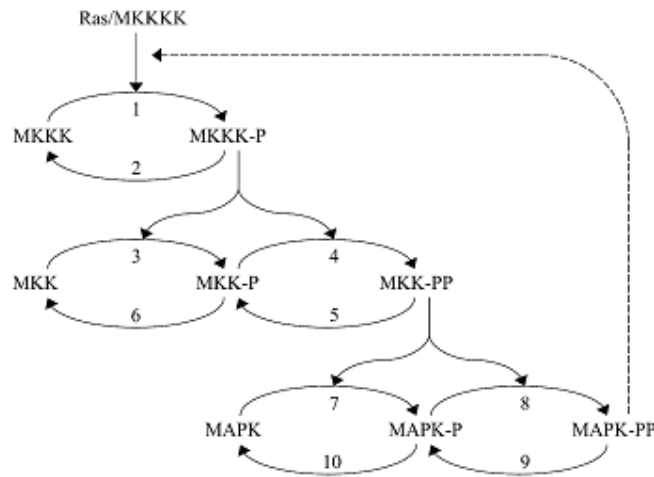
Figure 2: Kinetic scheme of the MAPK cascade

Simulation:
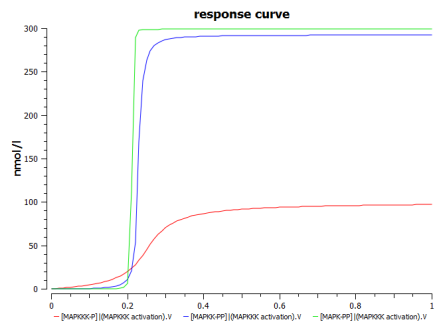
Open **kholodenko.cps** in COPASI and do the following:

- Run a time-course simulation of 400 seconds, using deterministic algorithm. (Tasks->Time Course).
- Plot the active forms of MAP kinase, MAP kinase kinase and MAP kinase kinase kinase in function of time.
  File: **kholodenko_timecourse.cps** (open in Copasi and run time-course to get the plot)
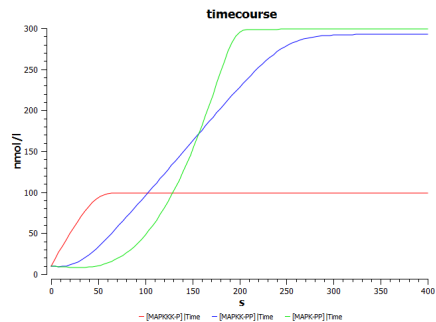
Sensitivity to signal:

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 MAP kinase, MAP kinase kinase and MAP kinase kinase kinase at steady state as a function of the initial activity of Ras (V1).

  File: **kholodenko_parameterscan.cps** (open in COPASI and run parameter scan to get the plot)

<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>

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

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

  If you have not added the new function "function_4_MAPKKK activation" as above, open **kholodenko_nf.cps** or import **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.
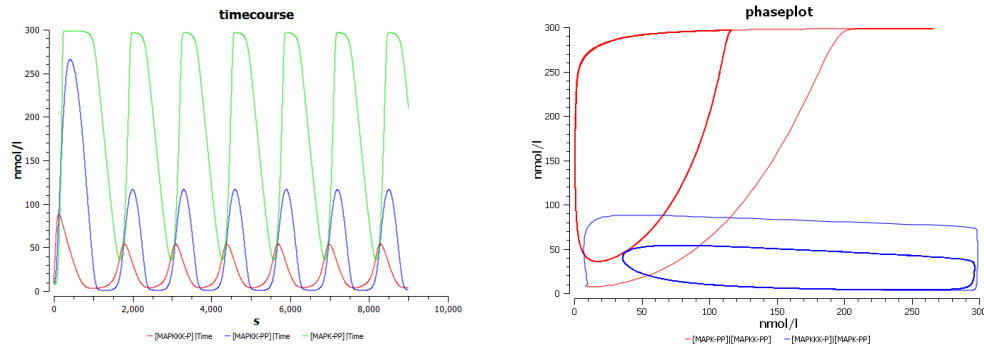
- Plot the active form of MAP kinase kinase kinase as a function of the active form of MAP kinase and the active form of MAP kinase as a function of the active form of MAP kinase kinase (plot in the right side).

  File: **kholodenko_nf_timecourse.cps** (open 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 MAP kinase and look at the change in

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the oscillation patterns (plot in the left side).

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

Files: **kholodenko_nf_parameterscan.cps** (open in COPASI and run parameter scan to get the plots below)
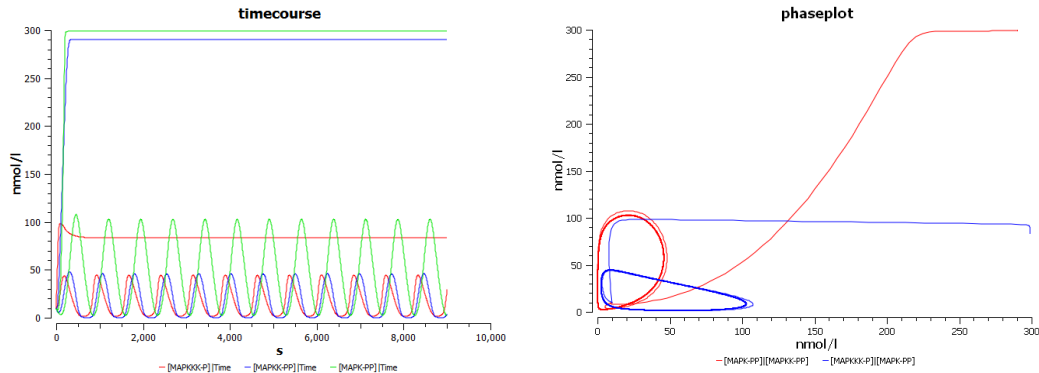
Additional Exercises (do the following if you have completed the above):

2. 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.**

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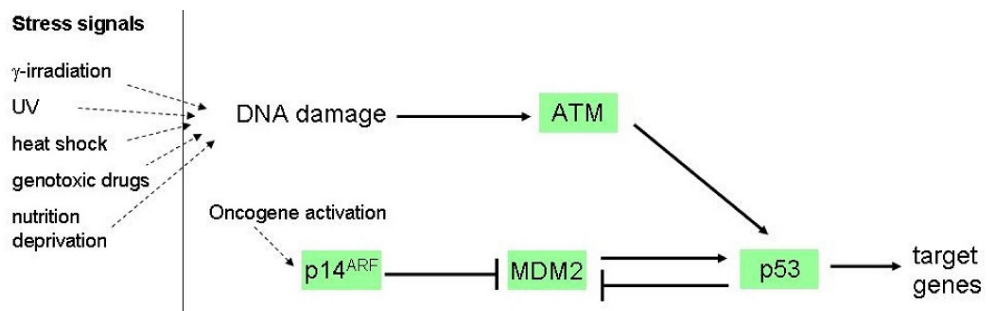- **p53 stabilization by ATM -** http://www.ebi.ac.uk/biomodels/BIOMD0000000188

Figure 3: Network diagram of p53 signaling pathway.

These are the models that have events in it.
Events: 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).

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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.
- 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.)
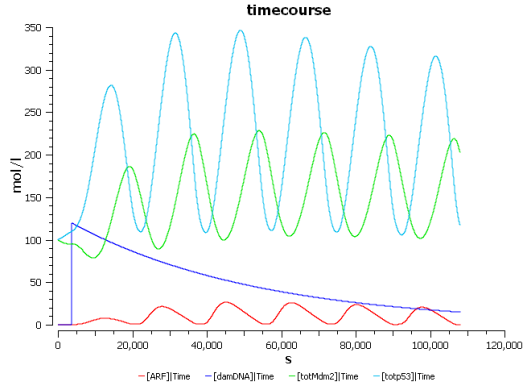
    File: **proctorARF_timecourse.cps** (open in COPASI and run time-course to get the plot)

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.
- 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-

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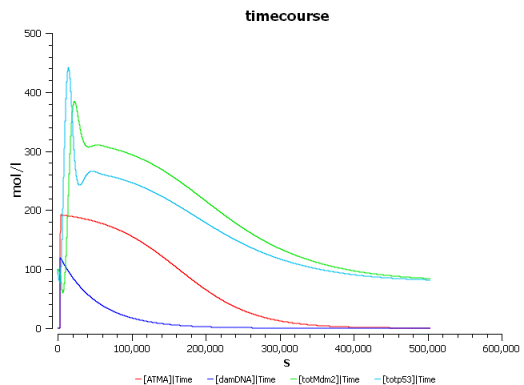
binding site of p53). Phosphorylation of the N-terminal end of p53 by kinases disrupts Mdm2-binding. In addition, Mdm2 also gets phosphorylated which also enhances the degradation of Mdm2. Thus, p53 gets stabilized and its transcriptional activity gets up-regulated in order to repair the DNA.)

File: **proctorATM_timecourse.cps** (open in COPASI and run time-course to get the plot)

3. Repressilator:

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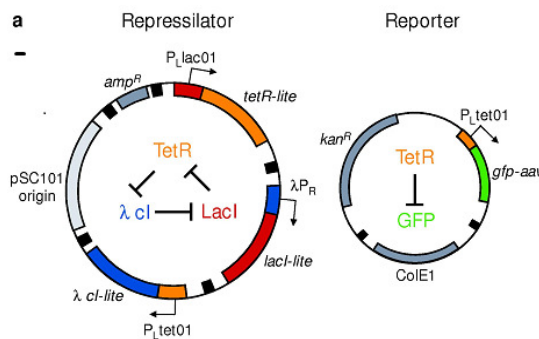(http://www.ebi.ac.uk/biomodels/BIOMD0000000012)

Figure 4: 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.

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, 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).
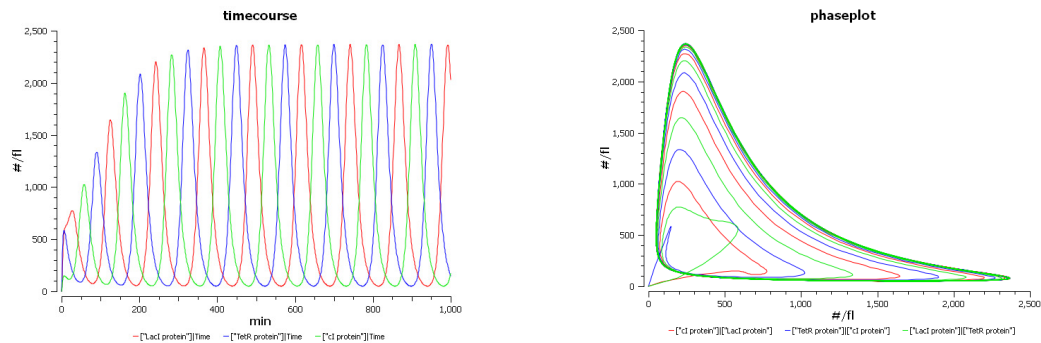
File: **elowitz_timecourse.cps** (open in COPASI and run time-course to get the above plots)

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Do it yourself and observe what happens by altering some key parameters:

- 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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