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To be submitted (preferably by e-mail to pencho.yordanov@bsse.ethz.ch), by Monday, April 2^{nd}, 2012. Please include the source files of your code.

Synthetic biology (Spring 2012): Mathematical models

Exercise 2. Toggle Switch

Consider the following mechanism:

$$G_1 \xrightarrow{k_1} G_1 + P_1 \tag{1}$$

$$G_1 + P_2 \underset{k_{c1}^-}{\overset{k_{c1}^+}{\rightleftharpoons}} \xi_1^I \tag{2}$$

$$G_2 \xrightarrow{k_2} G_2 + P_2 \tag{3}$$

$$G_2 + P_1 \underset{k_{c2}^-}{\overset{k_{c2}^+}{\rightleftharpoons}} \xi_2^{I_1} \tag{4}$$

$$\xi_2^{I_1} + P_1 \underset{k_{c3}^-}{\overset{k_{c3}^+}{\rightleftharpoons}} \xi_2^{I_2} \tag{5}$$

$$P_1 \xrightarrow{d_1} \emptyset \tag{6}$$

$$P_2 \xrightarrow{d_2} \emptyset \tag{7}$$

where:

- reactions (1) and (3) represent the expression of the proteins P_1 and P_2 starting from the genes G_1 and G_2, respectively,
- steps (6) and (7) account for the degradation of the proteins P_1 and P_2.

The expression of P_1 is repressed by the protein P_2, which binds to the promoter of G_1 forming a complex ξ_1^I. On the other hand, the expression of P_2 is repressed by protein P_1. In this case, there are two active binding sites in the promoter, leading to the formation of an intermediate complex $\xi_2^{I_1}$ which in turns binds to protein P_1 to form a complex $\xi_2^{I_2}$. Binding sites are cooperative provided that:

$$k_{c3}^{+} = \sigma k_{c2}^{+} \qquad \sigma >> 1$$

Let us consider, for simplicity, that $k_{c3}^{-} = k_{c2}^{-} = k_{c1}^{-}$ and $k_{c2}^{+} = k_{c1}^{+}$. For this mechanism, the number of reaction channels is $M = 10$, and the number of species involved is $N = 7$.
A deterministic model for the mechanism is given by the following set of ordinary differential equations:

$$\frac{d\mathbf{x}}{dt} = \mathcal{N}W \tag{8}$$

where $\mathbf{x} = ([G_1], [P_1], [\xi_1^I], [G_2], [P_2], [\xi_2^{I_1}], [\xi_2^{I_2}])^T$ is the concentration vector, $\mathcal{N}$ is the stoichiometric matrix $(N \times M)$ and W is the $(M \times 1)$ vector of reaction rates. The stoichiometric matrix contains the stoichiometric coefficients ν_{ij} of each species in every reaction step[1], and it can be constructed starting from the molecularity matrices associated to the reactions as follows:

$$\mathcal{N} = \beta - \alpha \tag{9}$$

where β_{ij} is the molecularity of the product species i in the reaction j and α_{ij} is the molecularity of the reactand species i in the reaction j. Note that both α_{ij} and β_{ij} are positive integers. For example, for reaction (1),

$$\alpha_1 = [1 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0],$$

$$\beta_1 = [1 \;\; 1 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0],$$

$$\nu_1 = [0 \;\; 1 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0].$$

where α_1^T is the first column of the reactand molecularity matrix α and ν_1^T is the first column of the stoichiometric matrix $\mathcal{N}$.
The reaction vector contains the rates for each of the reaction steps ($j = 1, \ldots, R$). Assuming mass action kinetics, the reaction rate associated to the j-th irreversible step will read:

$$W_j = k_j \prod_{i=1}^{N} x_i^{\alpha_{ij}} \tag{10}$$

1. For the *deterministic* simulation, use Matlab to integrate the system of ordinary differential equations given by (8). See the attached note with

[1]For this set up, we split reversible reactions into a forward and a backward step

syntax for creating an odefile and calling to ODE solvers. The volume of the cell under consideration is $10^{-14}L$, and the Avogadro number is $NA = 6,02 \cdot 10^{23}$ (molecules/mol). The species concentrations in nM are computed as follows:

$$c_i = 10^9 \cdot \frac{n_i}{NA \cdot V} \tag{11}$$

where n_i is the number of molecules of the species i.

a) Plot the evolution of the concentrations (expressed in nM) until $t = 500$ min, assuming the values of the parameters $S1$ in Table 1 starting from initial condition X_{01} in Table 2. Print the values of the level of proteins P_1 and P_2 at steady state (both in concentrations and in number of molecules).

b) Plot the evolution of the concentrations with initial condition given by X_{02}. Print the values of the level of proteins P_1 and P_2 at steady state (both in concentration and in number of molecules).

Is bistability detected for this set of parameters?.

Table I. Deterministic rate constants

	$S1$	units
k_1	100	$1/\text{min}$
k_{c1}^+	1	$1/(nM \cdot min)$
k_{c1}^-	1	$1/\text{min}$
k_2	1000	$1/\text{min}$
σ	100	-
d_1	6	$1/\text{min}$
d_2	2	$1/\text{min}$

Table II. Initial number of molecules

	X_{01}	X_{02}	X_{03}	X_{04}
G_1	1	1	10	10
P_1	20	500	20	500
ξ_1^I	0	0	0	0
G_2	1	1	10	10
P_2	20	500	20	500
$\xi_2^{I_1}$	0	0	0	0
$\xi_2^{I_2}$	0	0	0	0

2. For the *stochastic* simulation, use the routine SSA provided in the course web page. First:

a) Compute the set of values for the stochastic rate constants ($\hat{k}$) starting from the deterministic constants (k) given in Table 1. Take into account that:

- for unimolecular reactions

$$\hat{k}_j = k_j,$$

- for bimolecular (two species) reactions, and k_j given in $nM^{-1}min^{-1}$

$$\hat{k}_j = 10^9 \cdot k_j/(NA \cdot V).$$

b) Compute the values of the *propensities* associated to each reaction channel at t=0 for initial conditions X_{01}, X_{02}, X_{03} and X_{04}.

c) Provide the expression of the *state change vector* for every reaction channel.

d) Using the routine SSA provided in the course web page, run a stochastic simulation with initial condition X_{01} until $t = 500$ min (you might have to wait some time for the results).

e) Plot a histogram illustrating the probability distribution for the number of molecules of protein P_1 after $t > t_t$ where t_t is the time of the transient before reaching the steady state. See how to plot histograms in Matlab in the attached note.

3. (Optional). Perform tasks 1.a), 2.d) and 2.e) for initial conditions X_{03} and X_{04} and compare the results of the stochastic and deterministic simulations. Warning: Simulation might take more than two hours.

Odefile toggle.m

```
function dxdt = toggle(t,x,k)

 % reaction rates
 w = zeros(M,1);
 w(1) = k(1)*x(1);
 w(2) = ... ;
 w(3) = ... ;

      ...

 % stoichiometric matrix (N times M)
 N = [0  -1  1  0  0  0  0  0  0  0;
       ... ;
       ... ;
       ... ;
       ... ;
       ... ;
       ... ];

 dxdt = N*w;
```

Call to ODE solver from the main script

```
%
x0 = [];    % initial condition vector
tspan = [t0:ts:tf];  % specific times
                     % for the solution
k = [];     % parameter vector

% call to ODE solver, for example:
%  ode 45 (based  on Runge Kutta 4-5)
%  ode15s (for stiff problems)

[t,x] = ode45(@toggle,tspan,x0,[],k)

% plotting results
plot(t,x)

% plotting only x2 and x4 vs time
plot(t,x(:,2), t, x(:,4))
```

ssa.m

```
function [X, t] = ssa(c,t_fin,X0,v,alpha)

 % c: row vector of stochastic kinetic
 %   constants
 % t_fin: final time for the simulation
 % X0: column vector of initial conditions
 %   (number of molecules of every species
 %   at t=0)
 % v: (N times M) state change matrix
 % alpha:(N times M) molecularity matrix
 %   (contains the molecularities of
 %   the reactants for the M reaction channels)
```

Calling ssa from the main script

```
% Call to ssa
[X, t] = ssa(c,t_fin,X0,v,alpha)

% Plotting results
plot(t,X)
```

Plotting histograms

```
% Plotting histograms

t_t= ... ;  % define time of the transient

P1s = X(2,t_t:end);  % protein 1 for t>t_t

hh = histc(P1s,min(P1s):max(P1s));

bar(min(P1s):max(P1s),hh/sum(hh));
```