Manual: How to use Steadystateconc

S1. Instructions for Using 'Steadystateconc'

Steadystateconc is a software program for calculating true steady-state metabolite concentrations by the Runge-Kutta method and the S-system method. It may be downloaded for free from https://github.com/BioprocessdesignLab/Steadystateconc.git. The software is programmed in Microsoft Visual Studio 2015 (**Visual C++**). However, it should work with any other C++ language software. The instructions for using the software program are as follows.

1) Open the program folder. It contains the main execution file (**steadystateconc.sln**) and one folder (**steadystateconc**).

2) Open the **steadystateconc** folder. It contains another main execution file (**steadystateconc.cpp**) and two folders (**InputFile** and **OutputFile**).

3) Open the **InputFile** folder to define the necessary settings for the sensitivity calculations. It contains four files (**initial.dat**, **equation.h**, **rungekutta.dat**, and **print_format.dat**). They store the following information.

InputFile ……Folder including the data files necessary to execute COSMOS calculations.

|- **equation.h** ……	System description
|- **initial.dat** ……..	Data file defining the numbers of dependent and
|	independent variables, the numbers of fluxes, and
|	initial guesses for dependent variables and independent
|	variable values, and stoichiometric matrix values.
|- **integration.dat** ……..	File for integrating a differential equation model with
|	the Runge-Kutta method to obtain a better initial guess

4) Open the file **initial.dat** and input necessary values. Then save the data and close the file. As an example, consider the settings for a simple pathway metabolic reaction model shown in Fig. S1. Fig. S2 displays the settings for this model.

5) Open the file **equation.h** and describe your system in the space bounded by "//**********". Statements outside this range must not be changed.

4.1. Define variables and set their values in **double** type or **complex<double>** type in C++. Essentially any reasonable variable name can be used if it is defined here.

4.2. Describe the flux formulae that include the dependent variables, x[1] to x[n], and the independent variables, x[n+1] to x[n+m], in **complex<double>** C++ type.

4.3. Assign each individual flux *i* using the specified symbol "sv[i]= …….".
Save the settings and close the file. The settings are exemplified in Fig. S3 for the simple model.

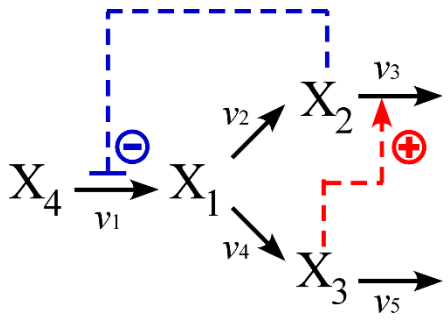

Fig. S1: Simple pathway metabolic reaction model

```
1                   // //(Flag for the execution of integration using numerical methods. 0; not
                      executed)

3 1 5               // (Total number of dependent variables n) (Total number of independent
                      variables m) (Total number of fluxes l)
10.0                // Value of first dependent variable x[1]
10.0                //          :          :
10.0                // Value of last dependent variable x[n]

3.0                 // Value of first independent variable x[n+1]
                    //          :          :
                    // Value of last dependent variable x[n+m]

                                          // Value of stoichiometric matrix N(X,v)=>
1     -1    0     -1    0     // N (x[1], sv[1]) .... N (x[1], sv[l])
0     1     -1    0     0     //          :          :
0     0     0     1     -1    // N (x[n], sv[1]) .... N (x[n], sv[l])
```

Fig. S2: Settings in the file **initial.dat** for the simple model shown in Fig. S1.

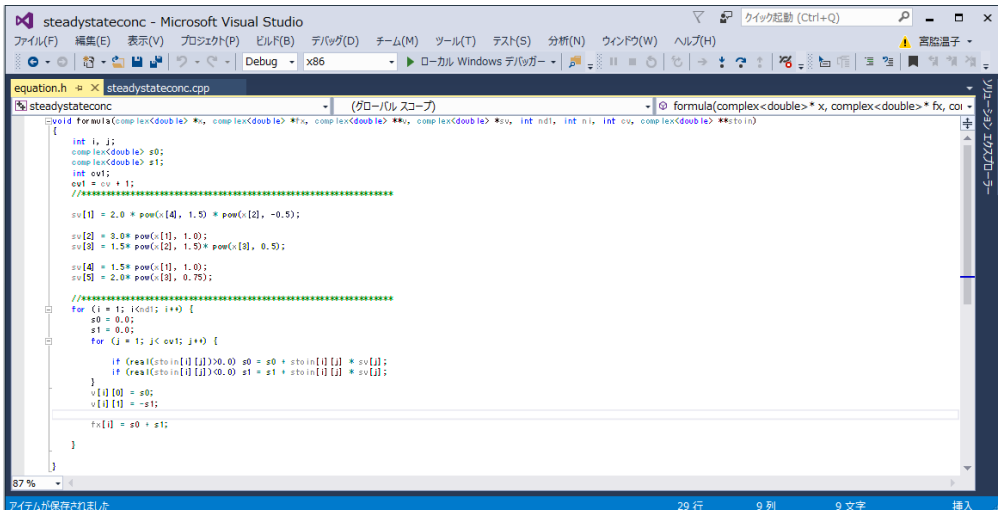

Fig. S3: Settings in the file **equation.h** for the simple model shown in Fig. S1.

6) The data file **integration.dat** is set for the Runge-Kutta method. The integration is executed when the first value in the data file **initial.dat** is set at 1. The format of the data is as follows.

0.0	// Start time
1000.0	// End of integration
0.1	// Sampling (storing) interval for calculated values (D=0.1)
100.0	// Value for devising the sampling time in order to increase the accuracy of calculated values (d=0.1/100.0=0.001).

7) Close the **InputFile** folder.

8) Open and run the main execution file **steadystateconc.sln**. The results are obtained very quickly.

9) Open the **OutputFile** folder to inspect the calculated results. It contains the following five files. All calculated values should be presented within round-off error.

OutputFile ……. Folder including the files generated as a result of the calculation.

|- **RK_Conc.dat** ……..….. Calculated data file, including pseudo steady-state
| concentrations calculated by Runge-Kutta method.
|- **Steady-state_Conc.dat** …… Calculated data file, including true steady-state
| metabolite concentrations.
|- **timecourse.dat** …… Data file for Runge-Kutta method.