**This file contains information regarding two types of models, both in Matlab and in PLAS, a convenient ODE solver that is freely available (<http://enzymology.fc.ul.pt/software/>). The explanation for the latter format are more detailed, as PLAS is not as widely known as Matlab.**

1. **Baseline Model:**

The MATLAB file corresponding to the PLAS file *Metal uptake.plc* is MATLAB\_PLANTS.m

1. **Baseline Model for Monte-Carlo Simulations**

The MATLAB files corresponding to the PLAS file *MC\_Arabidopsis.plc* are *MCplants.m* and *PlantDifEqlf.m*

***\*MATLAB files provide further comments within the code.***

**1. Baseline Model**

**MATLAB:**

The MATLAB file PLANTS.m contains code for all “What-If” simulations. It contains the ODEs, expressed in the format of all fluxes associated with each pool, which is followed by the symbolic definition of these fluxes, call of a ODE solver, and reporting instructions.

**PLAS:**

Download and implement PLAS, start it, and open the file *Metal uptake.plc*, which corresponds to the Matlab file above and contains the PLAS code for all “What-If” simulations. Although it is not mandatory, the file is subdivided into several sections for convenience. The first section shows the ODEs, again expressed in the format of influxes and effluxes associated with each pool, which is followed by the symbolic definition of these fluxes. The next section contains all initial values and parameter settings. The subsequent section contains alternative settings for the WBC19 mutant, Kan inhibition, etc. As an example, the first five lines of this section are comments:

Fe\_init: 5.9

Zn\_init: 1.8

VS1 \* 8

VS2 \* 0.3

VS4 \* 0.5

They indicate the measured initial values for the WBC19 mutant and one set of compensatory changes to VS1, VS2, and VS4, with which the model must be adjusted to simulate this scenario. For instance, either flux VS1 or its rate as1 is to be multiplied by 8, etc.

The final lines are definitions of Fe and Zn biomass, settings to model wild type (Kan =WBC = FRD3 = 1) or other scenarios, and the initial time, final time, and report interval.

The command

!! Fe\_biomass Zn\_biomass

is PLAS syntax instructing the program only to show the results for biomass.

The code is executed by clicking .

**2. Baseline Model for Monte-Carlo Simulations**

**MATLAB:** The MATLAB files *MCplants.m* and *PlantDifEqlf.m* contain code for Monte-Carlo simulations, where the two parameters as4 and g2 are randomized 1,000 times and the resulting residual errors are recorded.

**PLAS:**

The corresponding PLAS file is *MC\_Arabidopsis.plc*. Here, randomization is achieved through command lines like

as4 = rand[20] +5 //14

which randomizes as4 between 5 and 25; the slashes and 14 contain a comment, reminding us that the baseline value in the model was set to 14.

Similarly, the command

g2 = rand[1] - 1 //-.3

randomizes g2 between -1 and 0; its baseline value is -0.3.

Other additional lines in the code direct PLAS to compute the sums of squared errors for Fe and Zn at times 10 and 13 in comparison with the measured values. Finally, the PLAS-specific commands between

count1 = 1

and

end script

are syntax for executing the 10×10×10 = 1,000 Monte-Carlo iterations.

After running the file by clicking , the code is executed. Once completed, a window behind the code window shows the results in the form of a table:

Text

Description automatically generated

The entire table may be copied into Excel where, for instance, the sum of all four SSEs may be computed. The table is then sorted, *e.g*, by the total SSE. The initial part of the table of results is something like the following:

Chart, scatter chart

Description automatically generated

Of course, every simulation produces slightly different results, due to the random nature of the process. One may now plot, as an example, the different combinations of as4 and g2 that lead to very small SSE values, here defined as 2 times the optimal SSE. In this case, there is a clear, slightly curved relationship, and the settings of the model are roughly in the center of this line (14, -0.3).

Looking at the end of the Excel table shows that many combinations of as4 and g2 yield much worse SSEs:

Table

Description automatically generated

While as4 and g2 are still within the boundaries permitted by the simulation settings, the worst outcome of this particular simulation is an SSE of over 85, which is about 3,600 times as high as the best solution among those found in this simulation. Thus, the individual parameters are quite sensitive, but if their values happen to be close to the relationship shown above, they permit some variability without loss of quality of fit.