

This main script will read in the stoichiometry matrix of the metabolic network, reference steady state flux distribution and the standard Gibbs free energies of individual enzymatic reactions. The script calls for function *getExpandedA* and *getExpandedS* to expand the enzymatic reactions into elementary reactions. The reversibilities and kinetic parameters of individual elementary reactions are sampled under thermodynamic constrain by functions *samplingR* and *getKineticPara_Ther*. The script finally passes the sampled parameters to the core ODE function *ElementaryRxnsODE_2TMM_cons*.

The input data structure Net1 contains all the necessary inputs for running the program, and the required fields for Net1 are listed as following:

1. **S** – Stoichiometric matrix that consists of m metabolites (in rows) and n net reactions (in columns). Note there is currently a maximum of two reactants and three products that can participate in a single reaction. If a reaction involves more products or reactants, please split the reaction into multiple reactions. There are three rules of arranging the stiochiometric matrix: 1) the transport reactions out of the system are arranged at the end of the reaction columns; 2) the transport reactions into the system are arranged right before the transport out reactions; 3) the cofactors are arranged at the end of the metabolite rows. **[size: $m \times n$ matrix]**
2. **Sreg** – Regulation matrix in the same dimensions as S. For systems where no inhibition is assumed to take place, this should be entered as a matrix of zeros. For mixed inhibition enter a value of -3, for competitive inhibition a value of -1, and for uncompetitive inhibition a value of -2. These values should be entered in the row of the inhibitor metabolite and the column of the inhibited reaction. **[size: $m \times n$ matrix]**
3. **EnzName** – Enzyme names in the network. It can be array of name strings or numeric indexes. If two reactions are catalyzed by the same enzyme, give each of those reactions the same enzyme name or index number. **[size: $n \times 1$ vector]**
4. **Vin_index** – Index of which reactions represent transport reactions into the system, number values only. **[size: $u \times 1$ vector, where u is the number of uptake reactions]**
5. **Vout_index** – Index of which reactions represent transport reactions out of the system, number values only. **[size: $p \times 1$ vector, where p is the number of production reactions]**
6. **Vcof_index** – Index of the first cofactor for each pair of cofactors, number values only (e.g. if ATP & ADP are metabolites #11 and #12 in the S, place an 11 in this vector). **[size: $c \times 1$ vector, where c is the number of cofactor pools in the network]**

7. **SGFE** – Standard Gibbs free energies for each net reaction in kcal/mol. [**size: $n \times 1$ vector**]

8. **MetabRange** – Upper and lower bound for fold change in metabolite concentrations, used to calculate the thermodynamic constraints of the system. Default values for metabolites are 0.01 (lower) and 100 (upper), while for cofactors defaults are 1 (lower) and 1 (upper), since cofactor pools are tightly regulated. [**size: $m \times 2$ vector, first column lower bounds, second column upper bounds**]

9. **rVnet** – Net steady state flux distribution for the reference state. This must be calculated by the user such that all metabolites are balanced (this can be checked by multiplying S by $rVnet$ and obtaining a vector of zeros). [**size: $n \times 1$ vector**]

Optional Field :(can be left empty)

10. **GFERange** – Lower and upper bounds on the Gibbs free energies for each reaction. These can be inputted directly instead of being calculated from the standard energies and the range of metabolite concentrations. [**size: $n \times 2$ vector, first column lower bounds, second column upper bounds**]

The ensemble of x models can be constructed by repeating the core program functions: *samplingR*, *getKineticPara_Ther* and *ElementaryRxnsODE_2TMM_cons* x times. To perturb the enzyme concentrations, the variable *Econc* can be changed accordingly. Similarly, *Tconc* can be changed to simulate the concentration changes in transporter enzymes. If the system reaches steady state, the variable *vnet* stores the steady state flux distribution and *conc* contains the steady state metabolite profiles.

We have included an example Net1 input data structure for the following simple network:

