von Bertalanffy 1.1 tutorial

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1 Introduction

Reaction directionality in metabolic network reconstructions should be consistent with the second law of thermodynamics, which implies that reactions can only proceed in a direction associated with a negative (Legendre) transformed Gibbs energy ($\Delta_r G'$). $\Delta_r G'$ is reaction Gibbs energy at *in vivo* temperature (T), pH, ionic strength (I), electrical potential (ϕ) and metabolite concentrations (x).

In a typical metabolic reconstruction process, reaction directionality is assigned based on thermodynamic data such as experimentally measured equilibrium constants, whenever such data is available. Two problems frequently arise:

1. No thermodynamic data is available in the literature.
2. Thermodynamic data is available, but only for conditions that differ from *in vivo* conditions.

When these problems arise, reaction directionality is usually assigned *qualitatively*. In some cases it can be inferred from mechanistically similar reactions taking place at similar conditions. In other cases it is assigned based on modeling requirements. In the absence of any guiding principles, reaction directionality is set to reversible [1]. Qualitative directionality assignments can be viewed as model-driven hypotheses about *in vivo* reaction directionality.

Quantitative solutions to the aforementioned problems also exist. To solve the first problem, thermodynamic properties of metabolites and metabolic reactions can be estimated with computational methods such as the Group Contribution Method and IGERS [2, 3]. von Bertalanffy [4, 5] is a tool to solve the second problem.

von Bertalanffy 1.1 is a set of Matlab functions that enable *quantitative* assignment of reaction directionality in multicompartmental, genome-scale metabolic network reconstructions. Reaction directionality is assigned based on calculated ranges of feasible $\Delta_r G'$ at specified *in vivo* conditions. von Bertalanffy is freely available as part of the openCOBRA project at http://sourceforge.net/projects/opencobra/ [6].

2 Functionality

A simplified flow chart for von Bertalanffy is shown in Figure 2.1. The main inputs to von Bertalanffy are:

1. A metabolic reconstruction.

2. Metabolite standard Gibbs energies of formation ($\Delta_f G_i^0$), defined at standard conditions of T = 298.15 K, I = 0 M, and x = 1 M. $\Delta_f G_i^0$ can either be backcalculated from experimentally determined equilibrium constants, or estimated using the Group Contribution Method.

3. Acid dissociation constants (pK_a) for acidic and basic metabolites. The pK_a values can be estimated, for example with software from ChemAxon (Budapest, Hungary).

4. In vivo T , pH, I and ϕ for each compartment in the metabolic reconstruction. These values are obtained from experimental literature.

5. In vivo metabolite concentration ranges. Also obtained from literature if possible. Otherwise a broad default range is used.

von Bertalanffy comes with various support functions to format inputs. It also contains experimentally determined $\Delta_f G_i^0$ for some 200 common metabolites, and estimated $\Delta_f G_i^0$ for over 1000 metabolites. The main outputs from von Bertalanffy are:

1. Metabolite standard transformed Gibbs energies of formation ($\Delta_f G_i'^0$) at in vivo conditions and 1 M metabolite concentrations.

2. Metabolite transformed Gibbs energies of formation ($\Delta_f G_i'$) at in vivo conditions and in vivo metabolite concentrations.

3. Standard transformed and transformed reaction Gibbs energies ($\Delta_r G_k'^0$ and $\Delta_r G_k'$ respectively).

4. Reaction directionality.

Additional outputs include various directionality reports, and several figures depicting results.

References

[1] Thiele, I., and B. Ø. Palsson, 2010. A protocol for generating a high-quality genome-scale metabolic reconstruction. *Nat. Protoc.* 5:93–121.

[2] Jankowski, M. D., C. S. Henry, L. J. Broadbelt, and V. Hatzimanikatis, 2008. Group contribution method for thermodynamic analysis of complex metabolic networks. *Biophys. J.* 95:1487–1499.

[3] Rother, K., S. Hoffmann, S. Bulik, A. Hoppe, J. Gasteiger, and H. Holzhutter, 2010. IGERS: inferring Gibbs energy changes of biochemical reactions from reaction similarities. *Biophys. J.* 98:2478–2486.

[4] Fleming, R. M. T., I. Thiele, and H. P. Nasheuer, 2009. Quantitative assignment of reaction directionality in constraint-based models of metabolism: application to *Escherichia coli*. *Biophys. Chem.* 145:47–56.

[5] Fleming, R. M. T., and I. Thiele, 2011. von Bertalanffy 1.0: a COBRA toolbox extension to thermodynamically constrain metabolic models. *Bioinformatics* 27:142–143.

[6] Schellenberger, J., R. Que, R. M. T. Fleming, I. Thiele, J. D. Orth, A. M. Feist, D. C. Zielinski, A. Bordbar, N. E. R. Lewis, J. Kang, D. Hyduke, and B. Ø. Palsson, 2011. Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox v2.0. *Nat. Protoc.* In press.

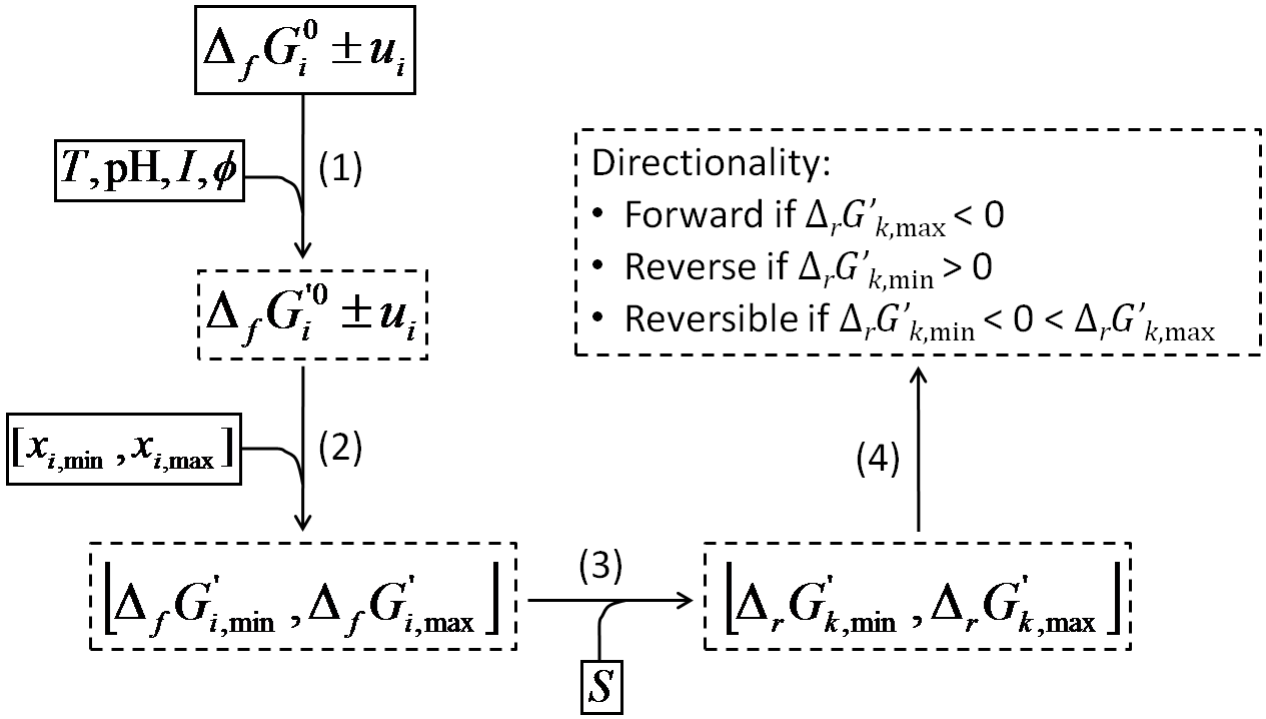


Figure 2.1: A simplified flow chart for the function von Bertalanffy. Inputs to the function have solid outlines and outputs have dashed outlines. **(1):** Metabolite standard Gibbs energies of formation ($\Delta_f G_i^0$) at $T = 298.15$ K, pH = 7, $I = 0$ M, $\phi = 0$ mV, and $x = 1$ M are transformed to *in vivo* T, pH, I and ϕ to obtain standard transformed Gibbs energies of formation ($\Delta_f G_i'^0$). $\Delta_f G_i^0$ are either obtained from experimental literature, or estimated using the Group Contribution Method. u_i is uncertainty in $\Delta_f G_i^0$. **(2):** *In vivo* metabolite concentration ranges are incorporated to yield feasible ranges of transformed Gibbs energies of formation ($\Delta_f G_i'$). The range for metabolite i is $\Delta_f G_i'^0 - u_i + RT\ln(x_{i,min}) = \Delta_f G'_{i,min} < \Delta_f G_i'^0 < \Delta_f G'_{i,max} = \Delta_f G_i'^0 + u_i + RT\ln(x_{i,max})$. **(3):** Feasible ranges of transformed reaction Gibbs energies ($\Delta_r G'_k$) are calculated for reactions in a metabolic reconstruction represented by the stoichiometric matrix $S \in \mathbb{Z}^{m,n}$. The range for reaction k is $\inf\{S_k^T \cdot \Delta_f G'\} = \Delta_r G'_{k,min} < \Delta_r G_k'^0 < \Delta_r G'_{k,max} = \sup\{S_k^T \cdot \Delta_f G'\}$, where $S_k \in \mathbb{Z}^{m,1}$ denotes a column from the stoichiometric matrix and $\Delta_r G_k'^0$ is the standard transformed reaction Gibbs energy at *in vivo* conditions when all metabolite concentrations are 1 M. **(4):** The directionality of each reaction is assigned based on its feasible $\Delta_r G'_k$ range.

3 Hands-on tutorial

In this tutorial you will use von Bertalanffy to quantitatively assign reaction directionality in the *E. coli* core model. Before you begin you should ensure that all necessary files are in your Matlab path, and that the COBRA toolbox has been initialized. In the Matlab command window type:

```
>> cd */SBSC2012/materials
>> addpath(genpath(pwd));
>> cd cobra
>> initCobraToolbox
```

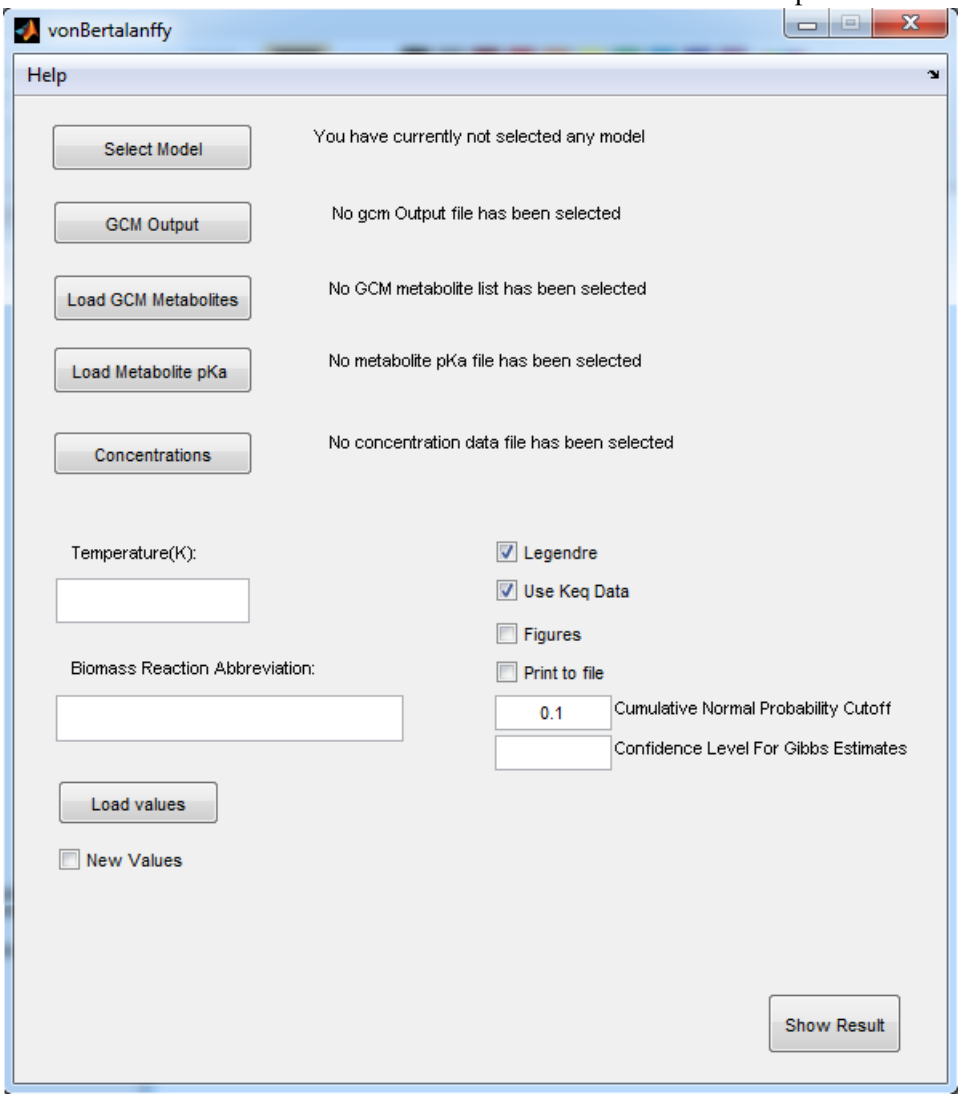
Substitute * in the first command with the path to your SBSC2012 folder.

3.1 Quantitative vs. qualitative directionality

1. We will run von Bertalanffy through a graphical user interface. First navigate to the folder where you saved the material for this tutorial (distributed via Dropbox), then type

>> vonBertalanffy

in the Matlab command window to start the interface. This should open the window shown below.

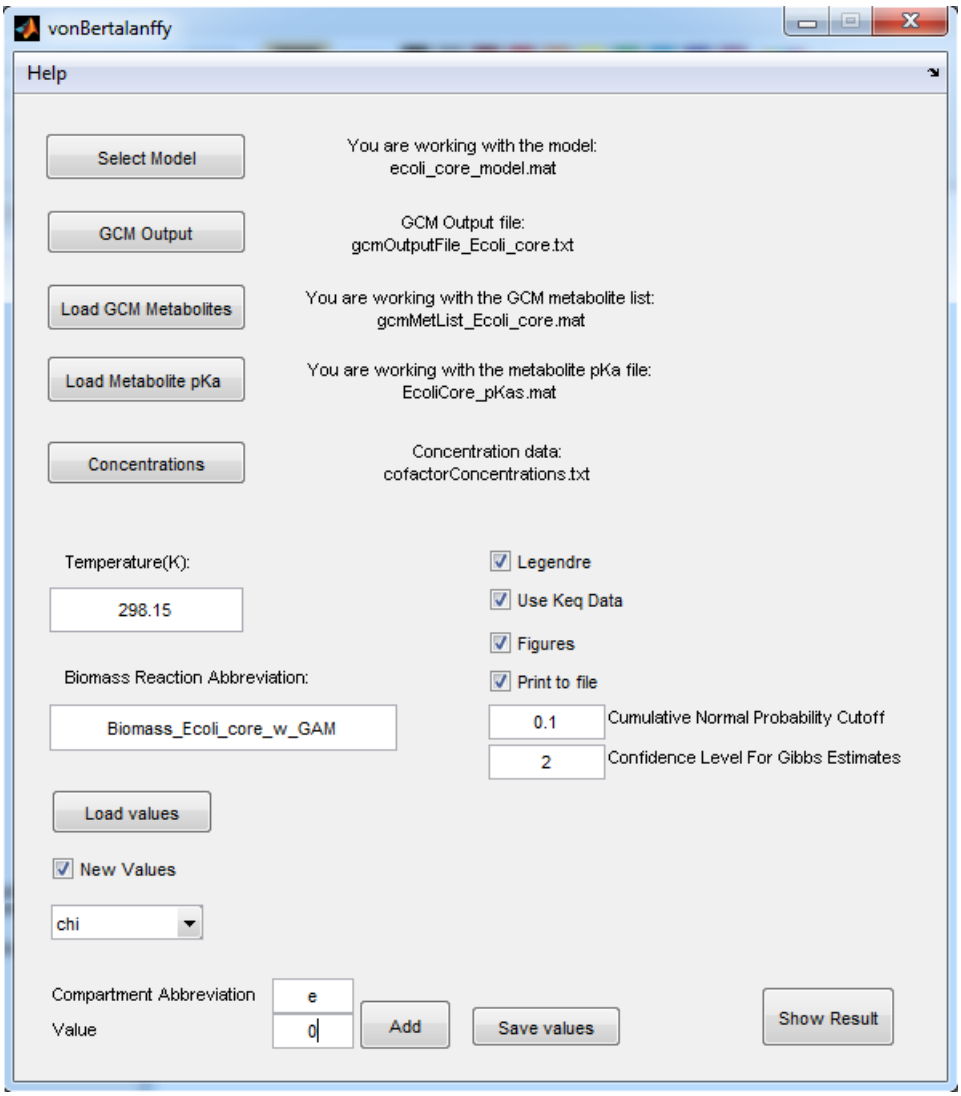


2. The next step is to load data stored in files into the Matlab workspace. Press "Select Model". A file explorer will open. Select "ecoli_core_model.mat" in the folder containing tutorial material.

3. Press the other four buttons and select the appropriate files according to the below table:

Button	File
GCM Output	gcmOutputFile_Ecoli_core.txt
Load GCM Metabolites	gcmMetList_Ecoli_core.mat
Metabolite pKa	EcoliCore_pKas.mat
Concentrations	cofactorConcentrations.txt

4. Set the temperature, objective and other variables as shown in the figure below



5. Set extracellular and cytosolic pH (pha), ionic strength (is) and electrical potential (chi). The figure above shows as example how extracellular electrical potential is set. Press "Add" after entering each variable. Once all variables have been set you can save them in a text file for later use. Values for all compartmental variables are given in the table below:

Variable	Cytosol (c)	Extracellular (e)
pH (pha)	7.7	6.5
Ionic strength (is)	0.25 M	0.25 M
Electrical potential (chi)	0 mV	0 mV

6. Press "Show Result" to run von Bertalanffy.

7. The main outputs from von Bertalanffy are:

   (a) Three Matlab variables, which can be viewed in the variable editor in Matlab:

      i. `modelT` is a structure similar to `model`, except with additional fields containing results from the thermodynamic analysis. The fields `metabolites` and `reactions` summarize thermodynamic results for metabolites and reactions, respectively.

ii. FBASolutions contains FBA results for the *E. coli* core model with qualitative and quantitative directionality, respectively, in the fields solutionRecon and solutionThermoRecon.

iii. directions contains boolean vectors with directionality results.

(b) Figures depicting major results.

(c) The directory "ecoli_core_thermoDirectionality". This directory contains various directionality reports in text files.

3.1.1 Assignments

Browse through the outputs and answer the following questions:

1. How many reactions had each of the following quantitative directionality assignments:

   (a) Forward?

   (b) Reverse?

   (c) Reversible?

2. List the number of reactions whose quantitative directionality fell into each of the following categories:

   (a) More constraining than qualitative directionality.

   (b) Less constraining than qualitative directionality.

   (c) In conflict with qualitative directionality.

3. Compare the growth rate of the *E. coli* core model before and after quantitative assignment of reaction directionality. List possible reasons for any difference.

3.2 Effects of environmental variables

1. Change extracellular electrical potential to 50 mV and rerun von Bertalanffy. Determine how this change affects directionality.

2. Change extracellular pH to 8 and determine how that affects directionality. Extracellular electrical potential should still be set to 50 mV.

3.3 Effects of experimental data

Set extracellular pH back to 6.5 and extracellular electrical potential back to 0 mV. Run von Bertalanffy again, but deselect the option "Use Keq Data" and replace "cofactorConcentrations.txt" with "noMetBounds.txt". This will eliminate all experimental data from the analysis and replace it with estimates.

1. Has the number of quantitatively reversible reactions changed? If so, then why?

2. Has the growth rate changed?