# Thermodynamically constrain a metabolic model

**Author: Ronan Fleming, University of Luxembourg**

**Reviewers:**

### INTRODUCTION

In flux balance analysis of genome scale stoichiometric models of metabolism, the principal constraints are uptake or secretion rates, the steady state mass conservation assumption and reaction directionality. Von Bertylanffy [1,4] is a set of methods for (i) quantitative estimation of thermochemical parameters for metabolites and reactions using the component contribution method [3], (ii) quantitative assignment of reaction directionality in a multi-compartmental genome scale model based on an application of the second law of thermodynamics to each reaction [2], (iii) analysis of thermochemical parameters in a network context, and (iv) thermodynamically constrained flux balance analysis. The theoretical basis for each of these methods is detailed within the cited papers.

### PROCEDURE

### Configure the environment

All the installation instructions are in a separate .md file named vonBertalanffy.md in docs/source/installation

With all dependencies installed correctly, we configure our environment, verfy all dependencies, and add required fields and directories to the matlab path.

```
initVonBertalanffy
```

## Select the model

This tutorial is tested for the E. coli model iAF1260 and the human metabolic model Recon3DModel_Dec2017. However, only the data for the former is provided within the COBRA Toolbox as it is used for testing von Bertylanffy, while Recon3D is not yet published and the data is not yet available. Having said this, the figures generated below are most suited to plotting results for Recon3D, so they may not be so useful for iAF1260.  The Recon3D example uses values from literature for input variables where they are available.

```
% modelName='iAF1260'; uncomment this line and comment the line below if you want to use the other model-  currently will not work without
modelName='Recon3DModel_Dec2017';
```

### Load a model

Load a model, and save it as the original model in the workspace, unless it is already loaded into the workspace.

```
clear model
global CBTDIR
modelFileName = [modelName '.mat']
modelDirectory = getDistributedModelFolder(modelFileName); %Look up the folder for the distributed Models.
modelFileName= [modelDirectory filesep modelFileName]; % Get the full path. Necessary to be sure, that the right model is loaded

switch modelName
    case 'iAF1260'
        model = readCbModel(modelFileName);
        if model.S(952, 350)==0
            model.S(952, 350)=1; % One reaction needing mass balancing in iAF1260
        end
        model.metCharges(strcmp('asntrna[c]', model.mets))=0; % One reaction needing charge balancing

    case 'Recon3DModel_Dec2017'
      model = readCbModel(modelFileName);
      model.csense(1:size(model.S,1),1)='E';
      %Hack for thermodynamics
      model.metFormulas{strcmp(model.mets,'h[i]')}='H';
      model.metFormulas(cellfun('isempty',model.metFormulas)) = {'R'};
      if isfield(model,'metCharge')
          model.metCharges = double(model.metCharge);
          model=rmfield(model,'metCharge');
      end
      modelOrig = model;
    otherwise
            error('setup specific parameters for your model')
end
```

### Set the directory containing the results

```
switch modelName
    case 'iAF1260'
        resultsPath=which('tutorial_vonBertalanffy.mlx');
        resultsPath=strrep(resultsPath,'/tutorial_vonBertalanffy.mlx','');
        resultsPath=[resultsPath filesep modelName '_results'];
        resultsBaseFileName=[resultsPath filesep modelName '_results'];
    case 'Recon3DModel_Dec2017'
        basePath='~/work/sbgCloud';
        resultsPath=[basePath '/programReconstruction/projects/recon2models/results/thermo/' modelName];
        resultsBaseFileName=[resultsPath filesep modelName '_' datestr(now,30) '_'];
    otherwise
        error('setup specific parameters for your model')
end
```

### Set the directory containing molfiles

```
switch modelName
    case 'iAF1260'
        molfileDir = 'iAF1260Molfiles';
    case 'Recon3DModel_Dec2017'
        molfileDir = [basePath '/data/molFilesDatabases/explicitHMol'];
        %molfileDir = [basePath '/programModelling/projects/atomMapping/results/molFilesDatabases/DBimplicitHMol'];
        %molfileDir = [basePath '/programModelling/projects/atomMapping/results/molFilesDatabases/DBexplicitHMol'];
    otherwise
        error('setup specific parameters for your model')
end
```

#### Set the thermochemical parameters for the model

```
switch modelName
    case 'iAF1260'
        T = 310.15; % Temperature in Kelvin
        compartments = ['c'; 'e'; 'p']; % Cell compartment identifiers
        ph = [7.7; 7.7; 7.7]; % Compartment specific pH
        is = [0.25; 0.25; 0.25]; % Compartment specific ionic strength in mol/L
        chi = [0; 90; 90]; % Compartment specific electrical potential relative to cytosol in mV
    case 'Recon3DModel_Dec2017'
        % Temperature in Kelvin
        T = 310.15;
        % Cell compartment identifiers
        compartments = ['c'; 'e'; 'g'; 'l'; 'm'; 'n'; 'r'; 'x';'i'];
        % Compartment specific pH
        ph = [7.2; 7.4; 6.35; 5.5; 8; 7.2; 7.2; 7; 7.2];
        % Compartment specific ionic strength in mol/L
        is = 0.15*ones(length(compartments),1);
        % Compartment specific electrical potential relative to cytosol in mV
        chi = [0; 30; 0; 19; -155; 0; 0; -2.303*8.3144621e-3*T*(ph(compartments == 'x') - ph(compartments == 'c'))/(96485.3365e-6); 0];
    otherwise
        error('setup specific parameters for your model')
end
```

#### Set the default range of metabolite concentrations

```
switch modelName
    case 'iAF1260'
        concMinDefault = 1e-5; % Lower bounds on metabolite concentrations in mol/L
        concMaxDefault = 0.02; % Upper bounds on metabolite concentrations in mol/L
        metBoundsFile=[];
    case 'Recon3DModel_Dec2017'
        concMinDefault=1e-5; % Lower bounds on metabolite concentrations in mol/L
        concMaxDefault=1e-2; % Upper bounds on metabolite concentrations in mol/L
        metBoundsFile=which('HumanCofactorConcentrations.txt');%already in the COBRA toolbox
    otherwise
        error('setup specific parameters for your model')
end
```

#### Set the desired confidence level for estimation of thermochemical parameters

The confidence level for estimated standard transformed reaction Gibbs energies is used to quantitatively assign reaction directionality.

```
switch modelName
    case 'iAF1260'
        confidenceLevel = 0.95;
        DrGt0_Uncertainty_Cutoff = 20; %KJ/KMol
    case 'Recon3DModel_Dec2017'
        confidenceLevel = 0.95;
        DrGt0_Uncertainty_Cutoff = 20; %KJ/KMol
    otherwise
        error('setup specific parameters for your model')
end
```

#### Prepare folder for results

```
if ~exist(resultsPath,'dir')
    mkdir(resultsPath)
end
cd(resultsPath)
```

#### Set the print level and decide to record a diary or not (helpful for debugging)

```
printLevel=2;

diary([resultsPath filesep 'diary.txt'])
```

## Setup a thermodynamically constrained model

#### Read in the metabolite bounds

```
setDefaultConc=1;
setDefaultFlux=0;
rxnBoundsFile=[];
model=readMetRxnBoundsFiles(model,setDefaultConc,setDefaultFlux,concMinDefault,concMaxDefault,metBoundsFile,rxnBoundsFile,printLevel);
```

#### Check inputs

```
model = configureSetupThermoModelInputs(model,T,compartments,ph,is,chi,concMinDefault,concMaxDefault,confidenceLevel);
```

```
Warning: Setting temperature to a value other than 298.15 K may introduce error, since enthalpies and heat capacities are not specified.
```

### Check elemental balancing of metabolic reactions

```
ignoreBalancingOfSpecifiedInternalReactions=1;
if ~exist('massImbalance','var')
    if isfield(model,'Srecon')
        model.S=model.Srecon;
    end
    % Check for imbalanced reactions
    fprintf('\nChecking mass and charge balance.\n');
    %Heuristically identify exchange reactions and metabolites exclusively involved in exchange reactions
    if ~isfield(model,'SIntMetBool')  ||  ~isfield(model,'SIntRxnBool') || ignoreBalancingOfSpecifiedInternalReactions
        %finds the reactions in the model which export/import from the model
        %boundary i.e. mass unbalanced reactions
        %e.g. Exchange reactions
        %     Demand reactions
        %     Sink reactions
        model = findSExRxnInd(model,[],printLevel);
    end

    if ignoreBalancingOfSpecifiedInternalReactions
        [nMet,nRxn]=size(model.S);
        ignoreBalancingMetBool=false(nMet,1);
        for m=1:nMet
%             if strcmp(model.mets{m},'Rtotal3coa[m]')
%                 pause(0.1);
%             end
            if ~isempty(model.metFormulas{m})
                ignoreBalancingMetBool(m,1)=numAtomsOfElementInFormula(model.metFormulas{m},'FULLR');
            end
        end
        ignoreBalancingRxnBool=getCorrespondingCols(model.S,ignoreBalancingMetBool,model.SIntRxnBool,'inclusive');
        SIntRxnBool=model.SIntRxnBool;
        model.SIntRxnBool=model.SIntRxnBool & ~ignoreBalancingRxnBool;
    end

    printLevelcheckMassChargeBalance=-1;  % -1; % print problem reactions to a file
    %mass and charge balance can be checked by looking at formulas
    [massImbalance,imBalancedMass,imBalancedCharge,imBalancedRxnBool,Elements,missingFormulaeBool,balancedMetBool]...
        = checkMassChargeBalance(model,printLevelcheckMassChargeBalance,resultsBaseFileName);
    model.balancedRxnBool=~imBalancedRxnBool;
    model.balancedMetBool=balancedMetBool;
    model.Elements=Elements;
    model.missingFormulaeBool=missingFormulaeBool;

    %reset original boolean vector
    if ignoreBalancingOfSpecifiedInternalReactions
        model.SIntRxnBool=SIntRxnBool;
    end
end
```

### Check that the input data necessary for the component contribution method is in place

```
model = setupComponentContribution(model,molfileDir);
```

```
Creating MetStructures.sdf from molfiles.
Percentage of metabolites without mol files: 100.0%
Converting SDF to InChI strings.
Estimating metabolite pKa values.
Assuming that metabolite species in model.metFormulas are representative for metabolites where pKa could not be estimated.
```

### Prepare the training data for the component contribution method

```
training_data = prepareTrainingData(model,printLevel);
```

```
Successfully added 3914 values from TECRDB
Successfully added 223 formation energies
Successfully added 13 redox potentials
Loading the InChIs for the training data from: /Users/zuck016/Projects/src/mycobratoolbox/src/analysis/thermo/componentContribution/cache/kegg_inc
Successfully created balanced training-data structure: 672 compounds and 4138 reactions
Loading the pKa values for the training data from: cache/kegg_pkas.mat
Mapping model metabolites to nist compounds
Creating group incidence matrix
python2 /Users/zuck016/Projects/src/mycobratoolbox/src/analysis/thermo/componentContribution/inchi2gv.py -s -i "InChI=1/C18H22O2/c1-18-9-8-14-13-5
Warning: getGroupVectorFromInchi did not succeed for: InChI=1/C18H22O2/c1-18-9-8-14-13-5-3-12(19)10-11(13)2-4-15(14)16(18)6-7-17(18)20/h3,5,10,14-
python2 /Users/zuck016/Projects/src/mycobratoolbox/src/analysis/thermo/componentContribution/inchi2gv.py -s -i "InChI=1/C18H24O2/c1-18-9-8-14-13-5
Warning: getGroupVectorFromInchi did not succeed for: InChI=1/C18H24O2/c1-18-9-8-14-13-5-3-12(19)10-11(13)2-4-15(14)16(18)6-7-17(18)20/h3,5,10,14-
Performing reverse transform
```

### Call the component contribution method

```
if ~isfield(model,'DfG0')
    [model,~] = componentContribution(model,training_data);
end
```

```
Running Component Contribution method
```

### Setup a thermodynamically constrained model

```
if ~isfield(model,'DfGt0')
    model = setupThermoModel(model,confidenceLevel);
end
```

```
Estimating standard transformed Gibbs energies of formation.

Estimating bounds on transformed Gibbs energies.
Additional effect due to possible change in chemical potential of Hydrogen ions for transport reactions.
Additional effect due to possible change in electrical potential for transport reactions.
```

### Generate a model with reactants instead of major microspecies

```
if ~isfield(model,'Srecon')
    printLevel_pHbalanceProtons=-1;

    model=pHbalanceProtons(model,massImbalance,printLevel_pHbalanceProtons,resultsBaseFileName);
end
```

### Determine quantitative directionality assignments

```
if ~exist('directions','var')
    fprintf('Quantitatively assigning reaction directionality.\n');
    [modelThermo, directions] = thermoConstrainFluxBounds(model,confidenceLevel,DrGt0_Uncertainty_Cutoff,printLevel);
end
```

```
Quantitatively assigning reaction directionality.
3/2382 reactions with DrGtMin=DrGtMax=0
4 inactive reactions (lb = ub = 0)
The following reactions have DrGtMax=DrGtMin=0:
H2Otex h2o[e]  <=> h2o[p]
H2Otpp h2o[p]  <=> h2o[c]
Htex h[e]  <=> h[p]
```

### Analyse thermodynamically constrained model

Choose the cutoff for probablity that reaction is reversible

```
cumNormProbCutoff=0.2;
```

Build Boolean vectors with reaction directionality statistics

```
[modelThermo,directions]=directionalityStats(modelThermo,directions,cumNormProbCutoff,printLevel);
```

```
3/2382 reactions with DrGtMin=DrGtMax=0
Qualitative internal reaction directionality:
      2077  internal reconstruction reaction directions.
      1520  forward reconstruction assignment.
         0  reverse reconstruction assignment.
       553  reversible reconstruction assignment.

Quantitative internal reaction directionality:
      2077  internal reconstruction reaction directions.
       549  of which have a thermodynamic assignment.
      1525  of which have no thermodynamic assignment.
        18  forward thermodynamic only assignment.
         1  reverse thermodynamic only assignment.
       530  reversible thermodynamic only assignment.

Qualitiative vs Quantitative:
       334  Reversible -> Reversible
         0  Reversible -> Forward
         1  Reversible -> Reverse
       215  Reversible -> Uncertain
        17  Forward -> Forward
         0  Forward -> Reverse
       195  Forward -> Reversible
      1308  Forward -> Uncertain
         0  Reverse -> Reverse
         0  Reverse -> Forward
         0  Reverse -> Reversible
         0  Reversible -> Uncertain

Breakdown of relaxation of reaction directionality, Qualitiative vs Quantitative:
       195  qualitatively forward reactions that are quantitatively reversible (total).
       162  of which are quantitatively reversible by range of dGt0. P(\Delta_{r}G^{\primeo}<0) > 0.7
         1  of which are quantitatively reversible by range of dGt0. 0.3< P(\Delta_{r}G^{\primeo}<0) < 0.7
        32  of which are quantitatively reversible by range of dGt0. P(\Delta_{r}G^{\primeo}<0) < 0.3
        71  of which are quantitatively forward by fixed dGr0t, but reversible by concentration alone (zero fixed DrGt0).
         0  of which are quantitatively reverse by dGr0t, but reversible by concentration (negative fixed DrGt0).
         0  of which are quantitatively forward by dGr0t, but reversible by concentration (positve fixed DrGt0).
         2  of which are quantitatively reverse by dGr0t, but reversible by concentration (uncertain negative DrGt0).
         2  of which are quantitatively forward by dGr0t, but reversible by concentration (uncertain positive DrGt0).
```

```
% directions    a structue of boolean vectors with different directionality
%               assignments where some vectors contain subsets of others
%
% qualtiative -> quantiative changed reaction directions
%   .forward2Forward
%   .forward2Reverse
%   .forward2Reversible
%   .forward2Uncertain
%   .reversible2Forward
%   .reversible2Reverse
%   .reversible2Reversible
%   .reversible2Uncertain
%   .reverse2Forward
%   .reverse2Reverse
%   .reverse2Reversible
%   .reverse2Uncertain
%   .tightened
%
% subsets of qualtiatively forward  -> quantiatively reversible
%   .forward2Reversible_bydGt0
%   .forward2Reversible_bydGt0LHS
%   .forward2Reversible_bydGt0Mid
%   .forward2Reversible_bydGt0RHS
%
%   .forward2Reversible_byConc_zero_fixed_DrG0
%   .forward2Reversible_byConc_negative_fixed_DrG0
%   .forward2Reversible_byConc_positive_fixed_DrG0
%   .forward2Reversible_byConc_negative_uncertain_DrG0
%   .forward2Reversible_byConc_positive_uncertain_DrG0
```

Write out reports on directionality changes for individual reactions to the results folder.

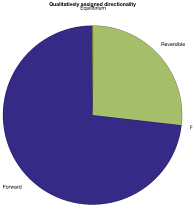
```
fprintf('%s\n','directionalityChangeReport...');
directionalityChangeReport(modelThermo,directions,cumNormProbCutoff,printLevel,resultsBaseFileName)
```

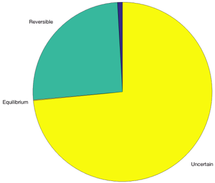
```
directionalityChangeReport...
```

Generate pie charts with proportions of reaction directionalities and changes in directionality

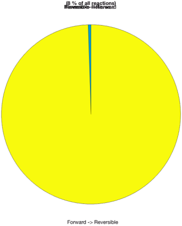
```
fprintf('%s\n','directionalityStatFigures...');
directionalityStatsFigures(directions,resultsBaseFileName)
```

```
directionalityStatFigures...
```

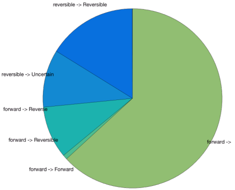




Qualtiative -> quantiative changed reaction directions
(8 % of all reactions)

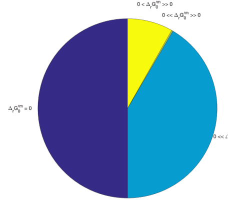


Qualtiative -> quantiative reaction directions

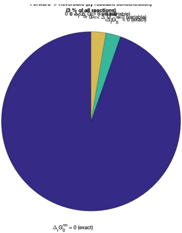


**Forward -> Reversible (by $\Delta_f G_0^{'m}$)**

**(16 % of all reactions)**



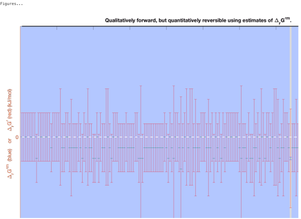
**Forward -> Reversible (by reactant concentration)**

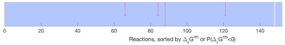


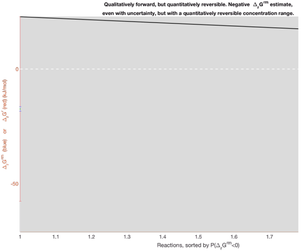
Generate figures to interpret the overall reasons for reaction directionality changes for the qualitatively forward now quantiatiavely reversible reactions

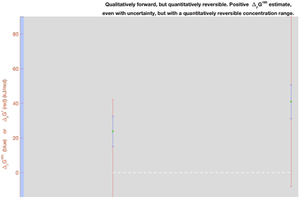
```
if any(directions.forward2Reversible)
    fprintf('%s\n','forwardReversibleFigures...');
    forwardReversibleFigures(modelThermo,directions,confidenceLevel)
end
```

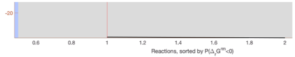
```
forwardReversibleFigures...
```



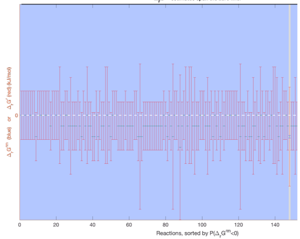



Qualitatively forward, but quantitatively reversible. Negative $\Delta_r G'^m$ estimate, even with uncertainty, but with a quantitatively reversible concentration range.


Qualitatively forward, but quantitatively reversible. Positive $\Delta_r G'^m$ estimate, even with uncertainty, but with a quantitatively reversible concentration range.



Qualitatively forward, but quantitatively reversible. The $\Delta_r G'^m$ estimates span the zero line.



Write out tables of experimental and estimated thermochemical parameters for the model

```
generateThermodynamicTables(modelThermo,resultsBaseFileName);
```

**REFERENCES**

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

[2] Haraldsdóttir, H. S., Thiele, I. & Fleming, R. M. T. Quantitative assignment of reaction directionality in a multicompartmental human metabolic reconstruction. Biophysical Journal 102, 1703–1711 (2012).

[3] Noor, E., Haraldsdóttir, H. S., Milo, R. & Fleming, R. M. T. Consistent Estimation of Gibbs Energy Using Component Contributions. PLoS Comput Biol 9, e1003098 (2013).

[4] Fleming, R. M. T. , Predicat, G., Haraldsdóttir, H. S., Thiele, I. von Bertalanffy 2.0 (in preparation).