Thermodynamically constrain a Recon3D

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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

The default COBRA Toolbox paths are automatically changed here to work on the new version of vonBertalanffy

```
aPath = which('initVonBertalanffy');
basePath = strrep(aPath,['vonBertalanffy' filesep 'initVonBertalanffy.m'],'');
addpath(genpath(basePath))
folderPattern=[filesep 'old'];
method = 'remove';
editCobraToolboxPath(basePath,folderPattern,method)
```

```
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/componentContribution/ol
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/directionalityReport/old
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/groupContribution/old
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/inchi/old
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/molFiles/old
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/protons/old
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/trainingModel/old
```

```
aPath = which('initVonBertalanffy');
basePath = strrep(aPath,['vonBertalanffy' filesep 'initVonBertalanffy.m'],'');
addpath(genpath(basePath))
folderPattern=[filesep 'new'];
method = 'add';
editCobraToolboxPath(basePath,folderPattern,method)
```

```
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/componentContribution/new
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/groupContribution/new
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/inchi/new
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/molFiles/new
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/protons/new
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/trainingModel/new
```

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
```

```
ChemAxon Marvin Beans is installed and working.
	linux-vdso.so.1 (0x00007ffdddfcf000)
	libc.so.6 => /lib/x86_64-linux-gnu/libc.so.6 (0x00007ff1adeef000)
	libopenbabel.so.5 => /usr/lib/libopenbabel.so.5 (0x00007ff1adc9f000)
	libstdc++.so.6 => /usr/lib/x86_64-linux-gnu/libstdc++.so.6 (0x00007ff1ada85000)
	libgcc_s.so.1 => /usr/local/bin/MATLAB/R2021a/sys/os/glnxa64/libgcc_s.so.1 (0x00007ff1ad86d000)
	/lib64/ld-linux-x86-64.so.2 (0x00007ff1ae10f000)
	libdl.so.2 => /lib/x86_64-linux-gnu/libdl.so.2 (0x00007ff1ad865000)
	libz.so.1 => /lib/x86_64-linux-gnu/libz.so.1 (0x00007ff1ad849000)
	libm.so.6 => /lib/x86_64-linux-gnu/libm.so.6 (0x00007ff1ad6fa000)
	libgomp.so.1 => /usr/lib/x86_64-linux-gnu/libgomp.so.1 (0x00007ff1ad6b5000)
	libpthread.so.0 => /lib/x86_64-linux-gnu/libpthread.so.0 (0x00007ff1ad692000)

babel must depend on the system libstdc++.so.6 not the one from MATLAB
Trying to edit the 'LD_LIBRARY_PATH' to make sure that it has the correct system path before the Matlab pa
The solution will be arch dependent
```

Select the model

This tutorial is tested for the E. coli model iAF1260 and the human metabolic model Recon3Dmodel. However, only the data for the former is provided within the COBRA Toolbox as it is used for testing von Bertylanffy. However, 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';
%modelName='Ec_iAF1260_flux1';
% uncomment this line and comment the line below if you want to use the other model-  c
modelName='Recon3DModel_301';
```

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']
```

```
modelFileName = 
'Recon3DModel_301.mat'
```

```


modelDirectory = getDistributedModelFolder(modelFileName); %Look up the folder for the 
modelFileName= [modelDirectory filesep modelFileName]; % Get the full path. Necessary t
```

```
switch modelName
    case 'Ec_iAF1260_flux1'
        modelFileName = [modelName '.xml']
        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[Cytosol]', model.mets))=0; % One reaction need

    case 'iAF1260'
        model = readCbModel(modelFileName);
        model.mets = cellfun(@(mets) strrep(mets,'_c','[c]'),model.mets,'UniformOutput'
        model.mets = cellfun(@(mets) strrep(mets,'_e','[e]'),model.mets,'UniformOutput'
        model.mets = cellfun(@(mets) strrep(mets,'_p','[p]'),model.mets,'UniformOutput'
        bool = strcmp(model.mets,'lipa[c]old[c]');
        model.mets{bool}='lipa_old_[c]';
        bool = strcmp(model.mets,'lipa[c]old[e]');
        model.mets{bool}='lipa_old_[e]';
        bool = strcmp(model.mets,'lipa[c]old[p]');
        model.mets{bool}='lipa_old_[p]';
        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 ch

    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;
   case 'Recon3DModel_301'
      model = readCbModel(modelFileName);
          %Hack for thermodynamics
      model.metFormulas(cellfun('isempty',model.metFormulas)) = {'R'};
      modelOrig = model;
    otherwise
            error('setup specific parameters for your model')
end
```

```
Each model.subSystems{x} has been changed to a character array.
```

Set the directory containing the results

```
switch modelName
    case 'Ec_iAF1260_flux1'
        resultsPath=which('tutorial_vonBertalanffy.mlx');
        resultsPath=strrep(resultsPath,'/tutorial_vonBertalanffy.mlx','');
        resultsPath=[resultsPath filesep modelName '_results'];
        resultsBaseFileName=[resultsPath filesep modelName '_results'];
    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/the
        resultsBaseFileName=[resultsPath filesep modelName '_' datestr(now,30) '_'];
    case 'Recon3DModel_301'
        basePath=['~' filesep 'work' filesep 'sbgCloud'];
        resultsPath=which('tutorial_vonBertalanffy.mlx');
        resultsPath=strrep(resultsPath,[filesep 'tutorial_vonBertalanffy.mlx'],'');
        resultsPath=[resultsPath filesep modelName '_results'];
        resultsBaseFileName=[resultsPath filesep modelName '_results_'];
    otherwise
        error('setup specific parameters for your model')
end
```

Set the directory containing molfiles

```
switch modelName
    case 'Ec_iAF1260_flux1'
        molFileDir = 'iAF1260Molfiles';
    case 'iAF1260'
        molFileDir = 'iAF1260Molfiles';
    case 'Recon3DModel_Dec2017'
        molFileDir = [basePath '/data/metDatabase/explicit/molFiles'];
        %molFileDir = [basePath '/programModelling/projects/atomMapping/results/molFile
        %molFileDir = [basePath '/programModelling/projects/atomMapping/results/molFile
    case 'Recon3DModel_301'
        ctfPath = [basePath filesep 'code' filesep 'fork-ctf'];
        % system(['git clone https://github.com/opencobra/ctf' ctfPath])
        molFileDir = [basePath filesep 'code' filesep 'fork-ctf' filesep 'mets' filesep
    otherwise
        molFileDir = [basePath '/code/fork-ctf/mets/molFiles'];
end
```

Set the thermochemical parameters for the model

```
switch modelName
    case 'Ec_iAF1260_flux1'
        T = 310.15; % Temperature in Kelvin
        compartments = {'Cytosol'; 'Extra_organism'; 'Periplasm'}; % Cell compartment i
        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 cyto
    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 cyto
    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')
    case 'Recon3DModel_301'
        % 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')
    otherwise
        error('setup specific parameters for your model')
end
```

Set the default range of metabolite concentrations

```
switch modelName
    case 'Ec_iAF1260_flux1'
        concMinDefault = 1e-5; % Lower bounds on metabolite concentrations in mol/L
        concMaxDefault = 0.02; % Upper bounds on metabolite concentrations in mol/L
        metBoundsFile=[];
    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 to
    case 'Recon3DModel_301'
        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 to
    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 'Ec_iAF1260_flux1'
        confidenceLevel = 0.95;
        DrGt0_Uncertainty_Cutoff = 20; %KJ/KMol
    case 'iAF1260'
        confidenceLevel = 0.95;
        DrGt0_Uncertainty_Cutoff = 20; %KJ/KMol
    case 'Recon3DModel_Dec2017'
        confidenceLevel = 0.95;
        DrGt0_Uncertainty_Cutoff = 20; %KJ/KMol
    otherwise
        confidenceLevel = -1;%bypass addition of uncertainty temporarily
        %confidenceLevel = 0.95;
        DrGt0_Uncertainty_Cutoff = 20; %KJ/KMol
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,concMaxD
```

```
Reading metabolite conc bounds from: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/ther
              adp[c]         1e-07        0.0019
              adp[m]        0.0026        0.0094
              amp[c]         1e-07        0.0012
              atp[c]       0.00129        0.0049
              atp[m]        0.0028        0.0204
              coa[c]      2.92e-05     0.0001168
              coa[m]        0.0022        0.0039
              na1[c]         1e-07         0.025
              na1[e]        0.1326        0.1554
              nad[c]    0.00010546     0.0007572
              nad[m]        0.0005        0.0075
             nadh[c]    9.2574e-07    0.00038294
             nadh[m]         1e-07        0.0011
             nadp[c]         1e-07    5.8284e-06
             nadp[m]         1e-07        0.0015
            nadph[c]         1e-07    0.00037523
            nadph[m]         1e-07        0.0042
              nh4[c]        0.0007        0.0009
               pi[c]         0.001        0.0063
              ppi[c]        0.0021        0.0076
              udp[g]       1.4e-06       0.00014
```

Check inputs

```
model = configureSetupThermoModelInputs(model,T,compartments,ph,is,chi,concMinDefault,c
```

```
Field metCompartments is missing from model structure. Attempting to create it.
Attempt to create field metCompartments successful.

Warning: Setting temperature to a value other than 298.15 K may introduce error, since enthalpies and heat
```

Add InChI to model

```
%[model, pKaErrorMets] = setupComponentContribution(model,molFileDir);
model = addInchiToModel(model, molFileDir, 'sdf', printLevel);
```

```
Creating MetStructures.sdf from molfiles.
Percentage of metabolites without mol files: 9.1%
Converting SDF to InChI strings.
5835 = number of model metabolites
5835 ... with mol files
0 ... without mol files
4949 ... with nonstandard inchi
886 ... without nonstandard inchi
108 ... compositie inchi removed
```

Add pseudoisomers to model

```
[model, nonphysicalMetBool, pKaErrorMetBool] = addPseudoisomersToModel(model, printLeve
```

```
Estimating metabolite pKa values.

ChemAxon's pKa calculator plugin returned an error for 2 metabolites:
    {'CE6252'      }    {'InChI=1/C5H3N4O3/c10-3-1-2(7-4(11)6-1)8-5(12)9-3/h(H3,6,7…'}
    {'pchol2ste_hs'}    {'InChI=1/C26H55NO7P/c1-5-6-7-8-9-10-11-12-13-14-15-16-17-1…'}

Assuming that metabolite species in model.metFormulas are representative for metabolites where pKa could n
5835 = number of model metabolites
217 = number of nonphysical model metabolites
3 = number of model metabolites with pKa error
```

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
    if ~isfield(model,'SIntMetBool')  ||  ~isfield(model,'SIntRxnBool') || ignoreBalanc
        %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.metFormula
            end
        end
        ignoreBalancingRxnBool=getCorrespondingCols(model.S,ignoreBalancingMetBool,mode
        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,missingFo
        = checkMassChargeBalance(model,printLevelcheckMassChargeBalance,resultsBaseFile
    model.balancedRxnBool=~imBalancedRxnBool;
    model.balancedMetBool=balancedMetBool;
    model.Elements=Elements;
    model.missingFormulaeBool=missingFormulaeBool;

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

```
Checking mass and charge balance.
Assuming biomass reaction is: biomass_maintenance
ATP demand reaction is not considered an exchange reaction by default. It should be mass balanced:
DM_atp_c_    h2o[c] + atp[c]     ->    h[c] + adp[c] + pi[c]
There are mass imbalanced reactions, see /home/rfleming/work/sbgCloud/code/fork-COBRA.tutorials/analysis/v
There are mass balanced, but charge imbalanced reactions, see /home/rfleming/work/sbgCloud/code/fork-COBRA
```

Create the thermodynamic training model

```
if 0
```

```
    %use previously generated training model
    aPath = which('driver_createTrainingModel.mlx');
    aPath = strrep(aPath,['new' filesep 'driver_createTrainingModel.mlx'],['cache' file
    load([aPath 'trainingModel.mat'])
else
    %recreate the trainingModel
    driver_createTrainingModel
end
```

```
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/componentContribution/ol
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/directionalityReport/old
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/groupContribution/old
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/inchi/old
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/molFiles/old
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/protons/old
removing: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/trainingModel/old
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/componentContribution/new
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/groupContribution/new
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/inchi/new
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/molFiles/new
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/protons/new
adding: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/analysis/thermo/trainingModel/new
ChemAxon Marvin Beans is installed and working.
    linux-vdso.so.1 (0x00007ffe937e7000)
    libc.so.6 => /lib/x86_64-linux-gnu/libc.so.6 (0x00007f25ad526000)
    libopenbabel.so.5 => /usr/lib/libopenbabel.so.5 (0x00007f25ad2d6000)
    libstdc++.so.6 => /usr/lib/x86_64-linux-gnu/libstdc++.so.6 (0x00007f25ad0bc000)
    libgcc_s.so.1 => /usr/local/bin/MATLAB/R2021a/sys/os/glnxa64/libgcc_s.so.1 (0x00007f25acea4000)
    /lib64/ld-linux-x86-64.so.2 (0x00007f25ad746000)
    libdl.so.2 => /lib/x86_64-linux-gnu/libdl.so.2 (0x00007f25ace9c000)
    libz.so.1 => /lib/x86_64-linux-gnu/libz.so.1 (0x00007f25ace80000)
    libm.so.6 => /lib/x86_64-linux-gnu/libm.so.6 (0x00007f25acd31000)
    libgomp.so.1 => /usr/lib/x86_64-linux-gnu/libgomp.so.1 (0x00007f25accec000)
    libpthread.so.0 => /lib/x86_64-linux-gnu/libpthread.so.0 (0x00007f25accc9000)

babel must depend on the system libstdc++.so.6 not the one from MATLAB
Trying to edit the 'LD_LIBRARY_PATH' to make sure that it has the correct system path before the Matlab pa
The solution will be arch dependent
Successfully added 3914 values from TECRDB
Successfully added 223 formation energies
Successfully added 13 redox potentials
mol2inchi: could not generate inchi for C00080
0 molecules converted
2 audit log messages

createInChIStruct: no molecule identifier in C00080
mol2inchi: could not generate inchi for C00080
0 molecules converted
2 audit log messages

mol2inchi: could not generate inchi for C00080
0 molecules converted
2 audit log messages

mol2inchi: could not generate inchi for C00080
0 molecules converted
2 audit log messages

mol2inchi: could not generate inchi for C00125
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C00125
mol2inchi: could not generate inchi for C00125
babel: Alias R was not chemically interpreted
```

```
mol2inchi: could not generate inchi for C00125
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00125
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00126
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C00126
mol2inchi: could not generate inchi for C00126
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00126
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00126
babel: Alias R was not chemically interpreted
mol2inchi: no annotation in C00225
createInChIStruct: no molecule identifier in C00225
mol2inchi: no annotation in C00225
mol2inchi: no annotation in C00225
mol2inchi: no annotation in C00225
mol2inchi: could not generate inchi for C00229
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C00229
mol2inchi: could not generate inchi for C00229
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00229
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00229
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00342
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C00342
mol2inchi: could not generate inchi for C00342
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00342
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00342
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00343
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C00343
mol2inchi: could not generate inchi for C00343
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00343
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C00343
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01003
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C01003
mol2inchi: could not generate inchi for C01003
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01003
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01003
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01194
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C01194
mol2inchi: could not generate inchi for C01194
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01194
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01194
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01209
```

```
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C01209
mol2inchi: could not generate inchi for C01209
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01209
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01209
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01277
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C01277
mol2inchi: could not generate inchi for C01277
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01277
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01277
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01281
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C01281
mol2inchi: could not generate inchi for C01281
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01281
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01281
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01299
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C01299
mol2inchi: could not generate inchi for C01299
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01299
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01299
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01931
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C01931
mol2inchi: could not generate inchi for C01931
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01931
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C01931
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02163
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C02163
mol2inchi: could not generate inchi for C02163
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02163
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02163
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02553
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C02553
mol2inchi: could not generate inchi for C02553
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02553
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02553
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02554
babel: Alias R was not chemically interpreted
```

```
createInChIStruct: no molecule identifier in C02554
mol2inchi: could not generate inchi for C02554
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02554
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02554
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02780
0 molecules converted
2 audit log messages

createInChIStruct: no molecule identifier in C02780
mol2inchi: could not generate inchi for C02780
0 molecules converted
2 audit log messages

mol2inchi: could not generate inchi for C02780
0 molecules converted
2 audit log messages

mol2inchi: could not generate inchi for C02780
0 molecules converted
2 audit log messages

mol2inchi: could not generate inchi for C02839
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C02839
mol2inchi: could not generate inchi for C02839
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02839
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02839
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02988
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C02988
mol2inchi: could not generate inchi for C02988
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02988
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02988
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02992
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C02992
mol2inchi: could not generate inchi for C02992
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02992
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C02992
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03127
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C03127
mol2inchi: could not generate inchi for C03127
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03127
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03127
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03511
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C03511
mol2inchi: could not generate inchi for C03511
```

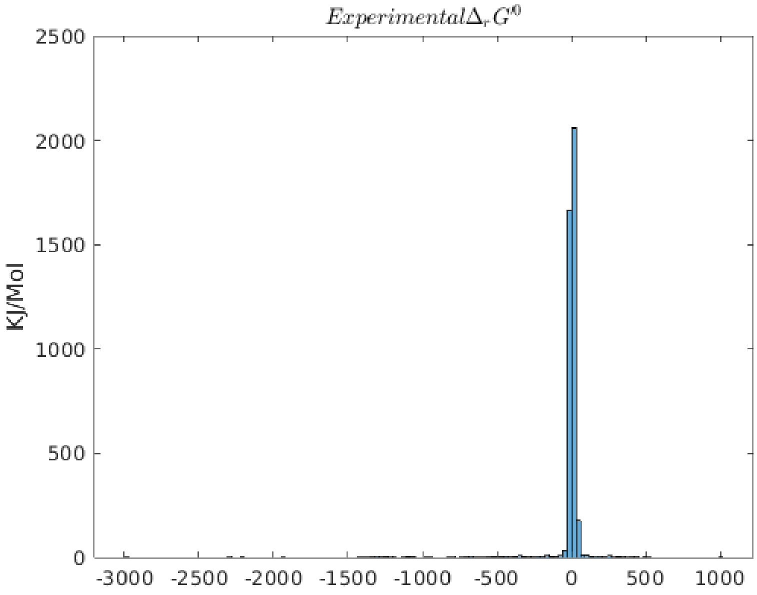
```
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03511
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03511
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03875
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C03875
mol2inchi: could not generate inchi for C03875
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03875
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03875
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03939
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C03939
mol2inchi: could not generate inchi for C03939
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03939
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C03939
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C04246
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C04246
mol2inchi: could not generate inchi for C04246
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C04246
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C04246
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C04618
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C04618
mol2inchi: could not generate inchi for C04618
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C04618
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C04618
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C04688
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C04688
mol2inchi: could not generate inchi for C04688
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C04688
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C04688
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C06020
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C06020
mol2inchi: could not generate inchi for C06020
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C06020
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C06020
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C06021
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C06021
mol2inchi: could not generate inchi for C06021
babel: Alias R was not chemically interpreted
```

```
mol2inchi: could not generate inchi for C06021
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C06021
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C06567
babel: Alias R was not chemically interpreted
createInChIStruct: no molecule identifier in C06567
mol2inchi: could not generate inchi for C06567
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C06567
babel: Alias R was not chemically interpreted
mol2inchi: could not generate inchi for C06567
babel: Alias R was not chemically interpreted
672 = number of model metabolites
657 ... with mol files
15 ... without mol files
627 ... with nonstandard inchi
45 ... without nonstandard inchi
0 ... compositie inchi removed

Estimating metabolite pKa values for training trainingModel...

 ...done.
There are mass imbalanced reactions, see /home/rfleming/work/sbgCloud/code/fork-COBRA.tutorials/analysis/v
Performing reverse Legendre transform
```

```
figure
histogram(trainingModel.DrGt0)
title('$Experimental \smallskip \Delta_{r} G^{\prime0}$','Interpreter','latex')
ylabel('KJ/Mol')
```



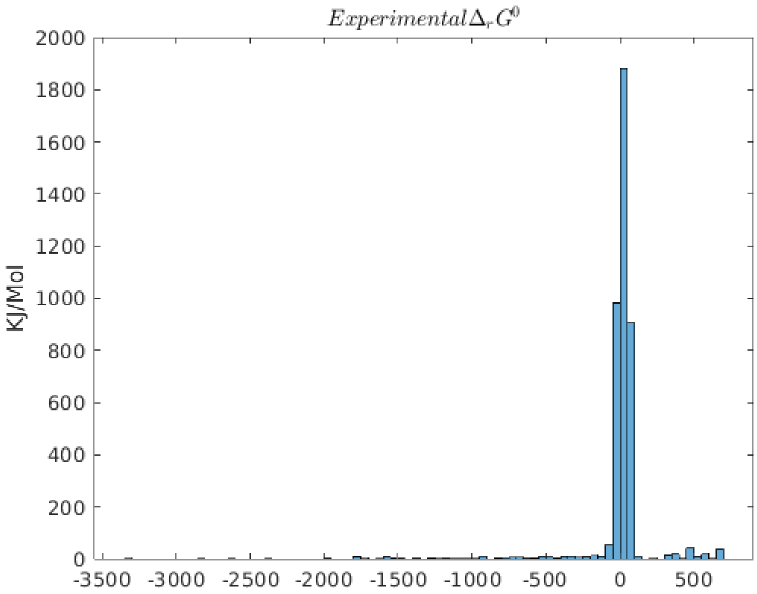
```
fprintf('%u%s\n',nnz(trainingModel.DrGt0==0),' = number of zero DrGt0, i.e. experimenta
```

```
35 = number of zero DrGt0, i.e. experimental apparent equilibrium constant equal to one
```

```
formulas = printRxnFormula(trainingModel,trainingModel.rxns(trainingModel.DrGt0==0));
```

```
TECRDB_79    C01101     <=>    C00231
TECRDB_244    C00041     <=>    C00133
TECRDB_580    C00002 + C00065 + C01650     <=>    C00013 + C00020 + C02553
TECRDB_698    C00003 + C00644     <=>    C00004 + C00085
TECRDB_733    C01101     <=>    C00231
TECRDB_815    C00002 + C00055     <=>    C00008 + C00112
TECRDB_1090    C00001 + C00006 + C00311     <=>    C00005 + C00026 + C00288
TECRDB_1272    C00001 + C06322     <=>    C06749
TECRDB_2030    C00041     <=>    C00133
TECRDB_2202    C00003 + C00579     <=>    C00004 + C00248
TECRDB_2339    C00063 + C00103     <=>    C00013 + C00501
TECRDB_2392    C01213     <=>    C00683
TECRDB_2584    C00041     <=>    C00133
TECRDB_2620    C00026 + C00041     <=>    C00022 + C00025
TECRDB_2621    C00002 + C01107     <=>    C00008 + C01143
TECRDB_2629    C00025     <=>    C00217
TECRDB_2639    C00002 + C00104     <=>    C00008 + C00081
TECRDB_2746    C00031     <=>    C00095
TECRDB_2791    C00010 + C00042 + C00044     <=>    C00009 + C00035 + C00091
TECRDB_2841    2 C00008     <=>    C00002 + C00020
TECRDB_2894    C00041     <=>    C00133
TECRDB_3608    C00031     <=>    C00095
TECRDB_3640    C00047     <=>    C00739
TECRDB_3803    C00636     <=>    C00275
TECRDB_3808    C00075 + C00103     <=>    C00013 + C00029
TECRDB_4052    C00041     <=>    C00133
TECRDB_4271    C00935     <=>    C00190
TECRDB_4375    C00123     <=>    C01570
TECRDB_4377    C00009 + C00299     <=>    C00106 + C00620
TECRDB_4398    C00026 + C00041     <=>    C00022 + C00025
TECRDB_4536    C00031     <=>    C00095
TECRDB_4537    C00031     <=>    C00095
FORM_C00023        <=>    C00023
FORM_C00034        <=>    C00034
FORM_C00080        <=>    C00080
```

```
figure
histogram(trainingModel.DrG0)
title('$Experimental \medskip \Delta_{r} G^{0}$','Interpreter','latex')
ylabel('KJ/Mol')
```



```
fprintf('%u%s\n',nnz(trainingModel.DrG0==0),' = number of zero DrG0. i.e. equilibrium c
```

```
16 = number of zero DrG0. i.e. equilibrium constant equal to one and same number of hydrogens on both side
```

```
formulas = printRxnFormula(trainingModel,trainingModel.rxns(trainingModel.DrG0==0));
```

```
TECRDB_79    C01101      <=>     C00231
TECRDB_244    C00041      <=>     C00133
TECRDB_733    C01101      <=>     C00231
TECRDB_1272    C00001 + C06322      <=>     C06749
TECRDB_2030    C00041      <=>     C00133
TECRDB_2392    C01213      <=>     C00683
TECRDB_2584    C00041      <=>     C00133
TECRDB_2629    C00025      <=>     C00217
TECRDB_2894    C00041      <=>     C00133
TECRDB_3640    C00047      <=>     C00739
TECRDB_4052    C00041      <=>     C00133
TECRDB_4271    C00935      <=>     C00190
TECRDB_4375    C00123      <=>     C01570
FORM_C00023        <=>     C00023
FORM_C00034        <=>     C00034
FORM_C00080        <=>     C00080
```

Create Group Incidence Matrix

Create the group incidence matrix (G) for the combined set of all metabolites.

```
save('data_prior_to_createGroupIncidenceMatrix')
```

```
%param.fragmentationMethod='manual';
param.fragmentationMethod='abinito';
param.printLevel=0;
param.modelCache=['autoFragment_' modelName];
param.debug=1;
param.radius=2;
```

```
combinedModel = createGroupIncidenceMatrix(model, trainingModel, param);
```

```
Creating group incidence matrix
There are 574 fragments unique to the training model.
There are 914 fragments in common between the training and test models.
There are 2659 fragments unique to the test model.
```

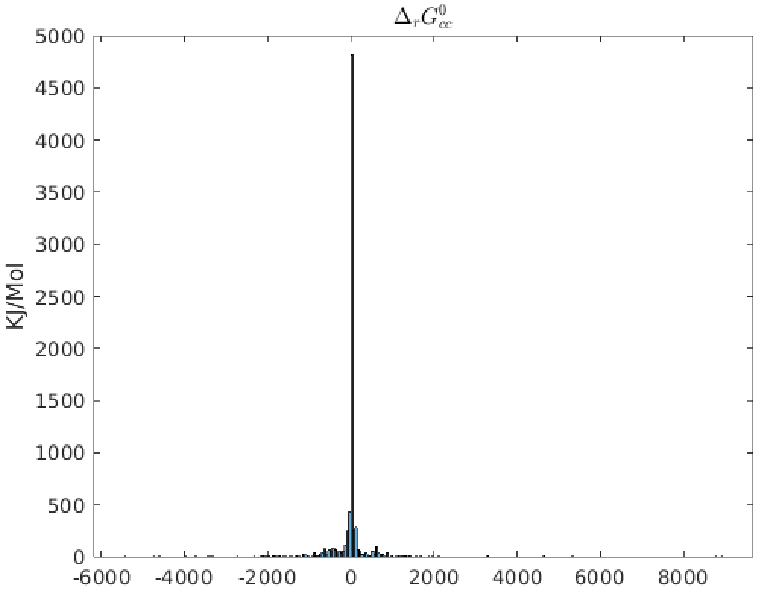
```
save('data_prior_to_componentContribution','model','combinedModel')
```

Apply component contribution method

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

```
Running Component Contribution method
```

```
figure
histogram(model.DrG0(~model.unconstrainedDrG0_cc))
title('$\Delta_{r} G^{0}_{cc}$','Interpreter','latex')
ylabel('KJ/Mol')
```



```
fprintf('%u%s\n',length(model.DrG0),' model reactions')
```

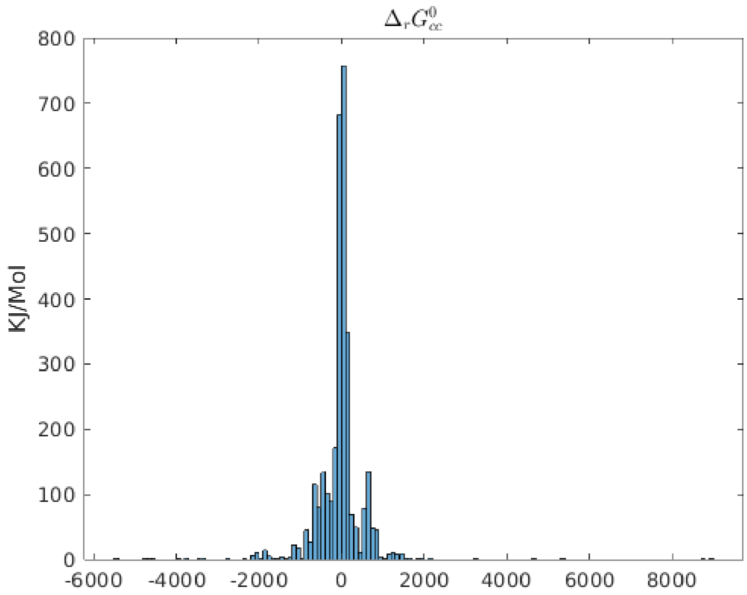
```
10600 model reactions
```

```
fprintf('%u%s\n',nnz(model.unconstrainedDrG0_cc),' of which have partially unconstraine
```

```
3147 of which have partially unconstrained groups in DrG0_cc
```

```
figure
model.transportRxnBool = transportReactionBool(model);
```

```
bool = model.SIntRxnBool & ~model.transportRxnBool & ~model.unconstrainedDrG0_cc;
histogram(model.DrG0(bool))
title('$\Delta_{r} G^{0}_{cc}$','Interpreter','latex')
ylabel('KJ/Mol')
```



```
fprintf('%u%s\n',length(model.DrG0),' model reactions')
```

```
10600 model reactions
```

```
fprintf('%u%s\n',nnz(model.unconstrainedDrG0_cc),' of which have partially unconstraine
```

```
3147 of which have partially unconstrained groups in DrG0_cc
```

```
ind=find(model.unconstrainedDrG0_cc);
formulas = printRxnFormula(model,model.rxns(ind(1:10)));
```

```
2AMACSULT	2amac[c] + nadph[c] + paps[c] 	->	nadp[c] + Lcyst[c] + pap[c] 
2DR1PP	h2o[c] + 2dr1p[c] 	->	pi[c] + drib[c] 
34DHPLACOX	h2o[c] + nad[c] + 34dhpac[c] 	->	2 h[c] + nadh[c] + 34dhpha[c] 
34DHPLACOX_NADP_	h2o[c] + nadp[c] + 34dhpac[c] 	<=>	2 h[c] + nadph[c] + 34dhpha[c] 
34DHXMANDACOX	h2o[c] + nad[c] + 34dhmald[c] 	->	2 h[c] + nadh[c] + 34dhoxmand[c] 
34DHXMANDACOX_NADP_	h2o[c] + nadp[c] + 34dhmald[c] 	<=>	2 h[c] + nadph[c] + 34dhoxmand[c] 
3AIBTm	2mop[m] + glu_L[m] 	<=>	akg[m] + 3aib[m] 
3HAO	o2[c] + 3hanthrn[c] 	->	h[c] + cmusa[c] 
3HBCDm	h2o[m] + b2coa[m] 	<=>	3hbcoa_R[m] 
3HLYTCL	h[c] + 34dhphe[c] 	->	co2[c] + dopa[c] 
```

Setup a thermodynamically constrained model

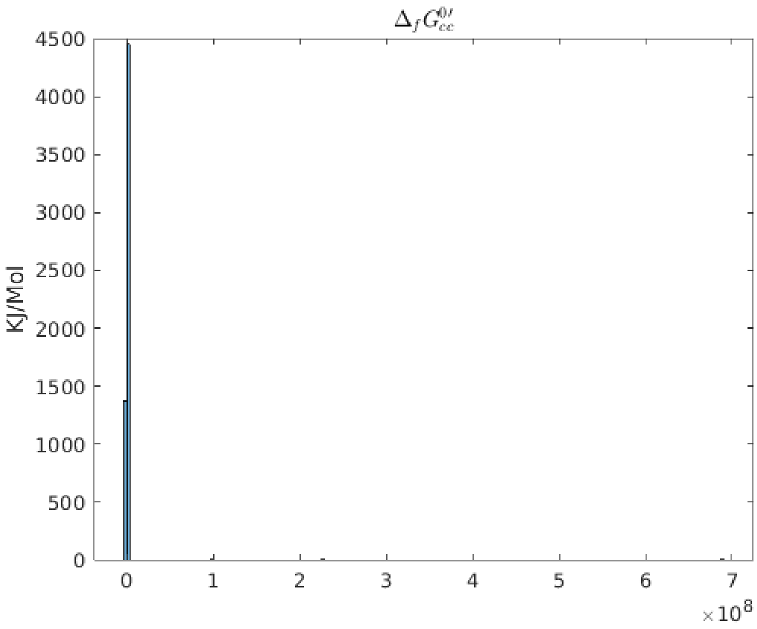
```
save('debug_prior_to_setupThermoModel')
```

```
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.
```

```
figure
histogram(model.DfGt0)
title('$\Delta_{f} G^{0\prime}_{cc}$','Interpreter','latex')
ylabel('KJ/Mol')
```



Generate a model with reactants instead of major microspecies

```
if ~isfield(model,'Srecon')
    printLevel_pHbalanceProtons=-1;
    model=pHbalanceProtons(model,massImbalance,printLevel_pHbalanceProtons,resultsBaseF
end
```

```
Warning: vonBertalanffy:pHbalanceProtons 'Hydrogen unbalanced reconstruction reactions exist!
```

Determine quantitative directionality assignments

```
if ~exist('directions','var') |  1
    fprintf('Quantitatively assigning reaction directionality.\n');
    [model, directions] = thermoConstrainFluxBounds(model,confidenceLevel,DrGt0_Uncerta
end
```

```
Quantitatively assigning reaction directionality.
9/10600 reactions with DrGtMin=DrGtMax~=0
4/10600 reactions with DrGtMin=DrGtMax=0
The following reactions have DrGtMax=DrGtMin=0:
H2Oter    h2o[c]     <=>    h2o[r]
```

```
H2Otn    h2o[n]     <=>    h2o[c]
Htr    h[c]     <=>    h[r]
HMR_1095    h[c]     <=>    h[n]
ACYP
```

Analyse thermodynamically constrained model

Choose the cutoff for probablity that reaction is reversible

```
cumNormProbCutoff=0.2;
```

Build Boolean vectors with reaction directionality statistics

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

```
9/10600 reactions with DrGtMin=DrGtMax~=0
4/10600 reactions with DrGtMin=DrGtMax=0
Qualitative internal reaction directionality:
      8791     internal reconstruction reaction directions.
      5208     forward reconstruction assignment.
         4     reverse reconstruction assignment.
      3579     reversible reconstruction assignment.

Quantitative internal reaction directionality:
      8791     internal reconstruction reaction directions.
      8036     of which have a thermodynamic assignment.
       751     of which have no thermodynamic assignment.
      1636     forward thermodynamic only assignment.
      1512     reverse thermodynamic only assignment.
      4888     reversible thermodynamic only assignment.

Qualitiative vs Quantitative:
      2525     Reversible -> Reversible
       347     Reversible -> Forward
       583     Reversible -> Reverse
       120     Reversible -> Uncertain
      1286     Forward -> Forward
       929     Forward -> Reverse
      2362     Forward -> Reversible
       631     Forward -> Uncertain
         1     Reverse -> Reverse
         3     Reverse -> Forward
         1     Reverse -> Reversible
         0     Reversible -> Uncertain

Breakdown of relaxation of reaction directionality, Qualitiative vs Quantitative:
      2362     qualitatively forward reactions that are quantitatively reversible (total).
      1183     of which are quantitatively reversible by range of dGt0. P(\Delta_{r}G^{\primeo}<0) > 0.7
         0     of which are quantitatively reversible by range of dGt0. 0.3< P(\Delta_{r}G^{\primeo}<0) <
      1179     of which are quantitatively reversible by range of dGt0. P(\Delta_{r}G^{\primeo}<0) < 0.3
        56     of which are quantitatively forward by fixed dGr0t, but reversible by concentration alone (
         0     of which are quantitatively reverse by dGr0t, but reversible by concentration (negative fix
         0     of which are quantitatively forward by dGr0t, but reversible by concentration (positve fixe
         0     of which are quantitatively reverse by dGr0t, but reversible by concentration (uncertain ne
         0     of which are quantitatively forward by dGr0t, but reversible by concentration (uncertain po
```

```
% 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...
```

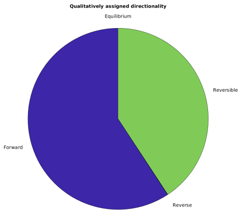
```
directionalityChangeReport(model,directions,cumNormProbCutoff,printLevel,resultsBaseFil
```

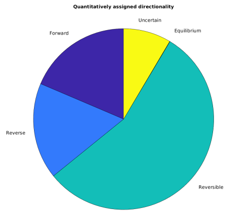
Generate pie charts with proportions of reaction directionalities and changes in directionality

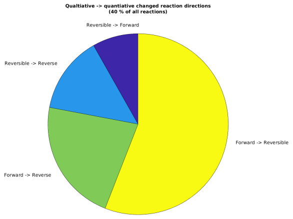
```
fprintf('%s\n','directionalityStatFigures...');
```

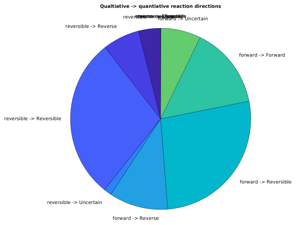
```
directionalityStatFigures...
```

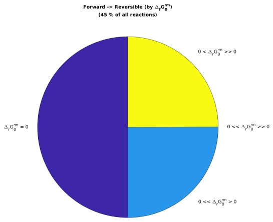
```
directionalityStatsFigures(directions,resultsBaseFileName)
```

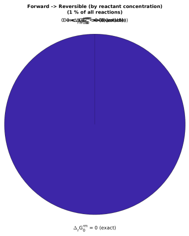



Quantitatively assigned directionality


Qualtiative -> quantiative changed reaction directions
(40 % of all reactions)


Qualtiative -> quantiative reaction directions

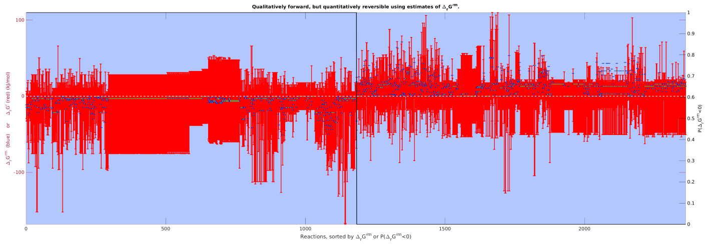

Forward -> Reversible (by $\Delta_f G_0'^m$)
(45 % of all reactions)

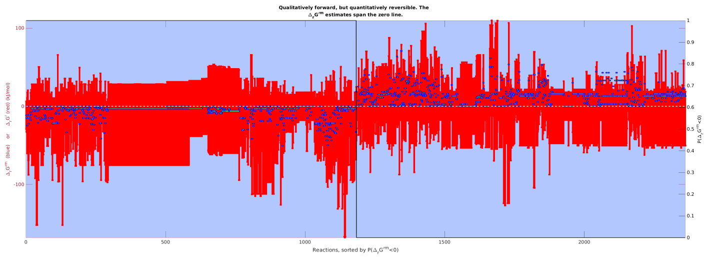

Forward -> Reversible (by reactant concentration)
(1 % of all reactions)

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(model,directions,confidenceLevel)
end
```

```
forwardReversibleFigures...
```





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

```
generateThermodynamicTables(model,resultsBaseFileName);
save([datestr(now,30) '_' modelName '_thermo'],'model')
save([datestr(now,30) '_vonB_tutorial_complete'])
```

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).