

Thermodynamically constrain iAF1260

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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 Bertalanffy [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, verify 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. However, only the data for the former is provided within the COBRA Toolbox as it is used for testing von Bertalanffy. 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- currently will not work without  
changes  
%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
modelName = [modelName '.mat']
```

```
modelName =
'IAF1260.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 '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 needing charge balancing

    case 'iAF1260'
        model = readCbModel(modelFileName);
        model.mets = cellfun(@(mets)
strrep(mets, '_c', '[c]'), model.mets, 'UniformOutput', false);
        model.mets = cellfun(@(mets)
strrep(mets, '_e', '[e]'), model.mets, 'UniformOutput', false);
        model.mets = cellfun(@(mets)
strrep(mets, '_p', '[p]'), model.mets, 'UniformOutput', false);
        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 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

```

Each `model.subSystems{x}` is a character array, and this format is retained.

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/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 'Ec_iAF1260_flux1'
    molfileDir = 'iAF1260Molfiles';
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 'Ec_iAF1260_flux1'
    T = 310.15; % Temperature in Kelvin
    compartments = {'Cytosol'; 'Extra_organism'; 'Periplasm'}; % 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 '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 '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 toolbox
        otherwise
            error('setup specific parameters for your model')
    end
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
        error('setup specific parameters for your model')
end
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);
```

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

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
end

```

Checking mass and charge balance.

Assuming biomass reaction is: BIOMASS_Ec_iAF1260_core_59p81M

ATP maintenance reaction is not considered an exchange reaction by default. It should be mass balanced:

ATPM atp[c] + h2o[c] -> adp[c] + h[c] + pi[c]

There are mass imbalanced reactions, see </home/rfleming/work/sbgCloud/code/fork-COBRA.tutorials/analysis/v>

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: /home/rfleming/work/sbgCloud/code/fork-cobratoolbox/src/ana  
Successfully created balanced training-data structure: 672 compounds and 3061 reactions  
Loading the pKa values for the training data from: cache/kegg_pkas.mat  
Mapping model metabolites to nist compounds  
Creating group incidence matrix  
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, result  
sBaseFileName);  
end
```


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

Determine quantitative directionality assignments

```
if ~exist('directions','var')
    fprintf('Quantitatively assigning reaction directionality.\n');
    [modelThermo, directions] =
thermoConstrainFluxBounds(model,confidenceLevel,DrGt0_Uncertainty_Cutoff,prin
tLevel);
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]
H2Otp    h2o[p]    <=>    h2o[c]
Htex      h[e]      <=>    h[p]
```

Analyse thermodynamically constrained model

Choose the cutoff for probability that reaction is reversible

```
cumNormProbCutoff=0.2;
```

Build Boolean vectors with reaction directionality statistics

```
[modelThermo,directions]=directionalityStats(modelThermo,directions,cumNormPr
obCutoff,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.
   17    forward thermodynamic only assignment.
    0    reverse thermodynamic only assignment.
  532    reversible thermodynamic only assignment.
```

```
Qualitative vs Quantitative:
 335    Reversible -> Reversible
    0    Reversible -> Forward
    0    Reversible -> Reverse
 215    Reversible -> Uncertain
   16    Forward -> Forward
    0    Forward -> Reverse
  196    Forward -> Reversible
 1308    Forward -> Uncertain
    0    Reverse -> Reverse
    0    Reverse -> Forward
    0    Reverse -> Reversible
```

0 Reversible -> Uncertain

Breakdown of relaxation of reaction directionality, Qualitative vs Quantitative:

```
196 qualitatively forward reactions that are quantitatively reversible (total).
136 of which are quantitatively reversible by range of dGt0.  $P(\Delta_r G^{\prime} < 0) > 0.7$ 
1 of which are quantitatively reversible by range of dGt0.  $0.3 < P(\Delta_r G^{\prime} < 0) < 0.7$ 
59 of which are quantitatively reversible by range of dGt0.  $P(\Delta_r G^{\prime} < 0) < 0.3$ 
15 of which are quantitatively forward by fixed dGr0t, but reversible by concentration alone (negative fixed dGr0t)
0 of which are quantitatively reverse by dGr0t, but reversible by concentration (negative fixed dGr0t)
0 of which are quantitatively forward by dGr0t, but reversible by concentration (positive fixed dGr0t)
3 of which are quantitatively reverse by dGr0t, but reversible by concentration (uncertain negative fixed dGr0t)
2 of which are quantitatively forward by dGr0t, but reversible by concentration (uncertain positive fixed dGr0t)
```

```
% directions      a structure of boolean vectors with different directionality
%                  assignments where some vectors contain subsets of others
%
% qualitative -> quantitative changed reaction directions
% .forward2Forward
% .forward2Reverse
% .forward2Reversible
% .forward2Uncertain
% .reversible2Forward
% .reversible2Reverse
% .reversible2Reversible
% .reversible2Uncertain
% .reverse2Forward
% .reverse2Reverse
% .reverse2Reversible
% .reverse2Uncertain
% .tightened
%
% subsets of qualitatively forward -> quantitatively 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...
```

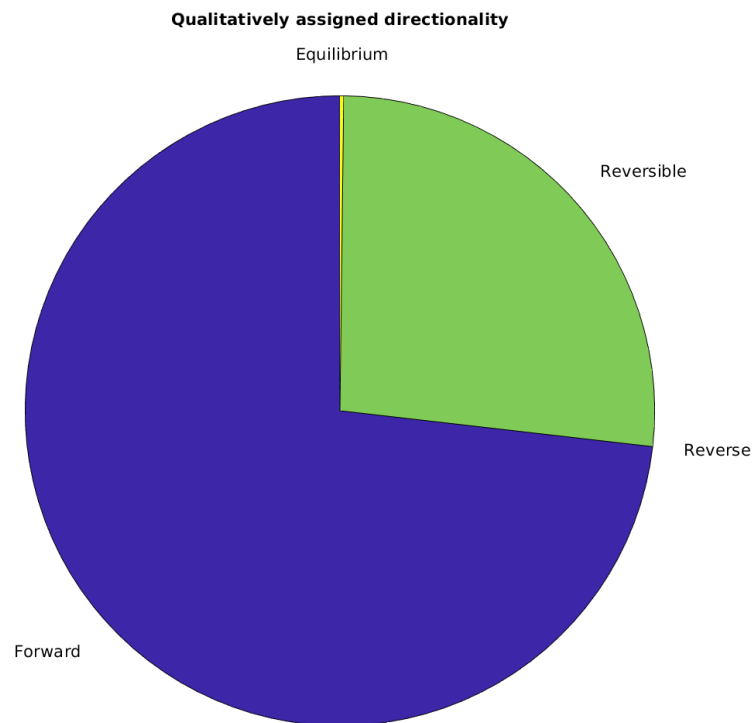
```
directionalityChangeReport(modelThermo,directions,cumNormProbCutoff,printLevel,resultsBaseFileName)
```

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

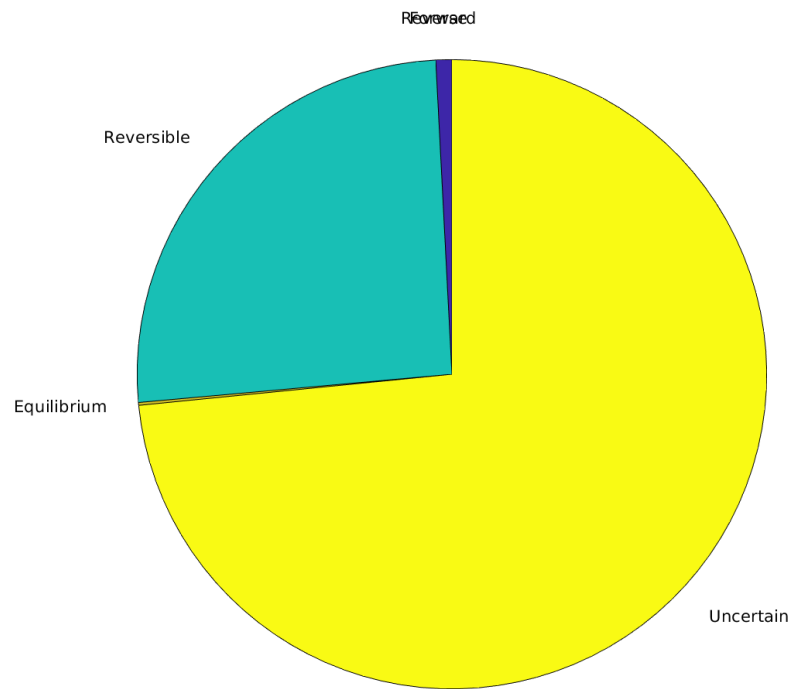
```
fprintf('%s\n','directionalityStatFigures...');
```

```
directionalityStatFigures...
```

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

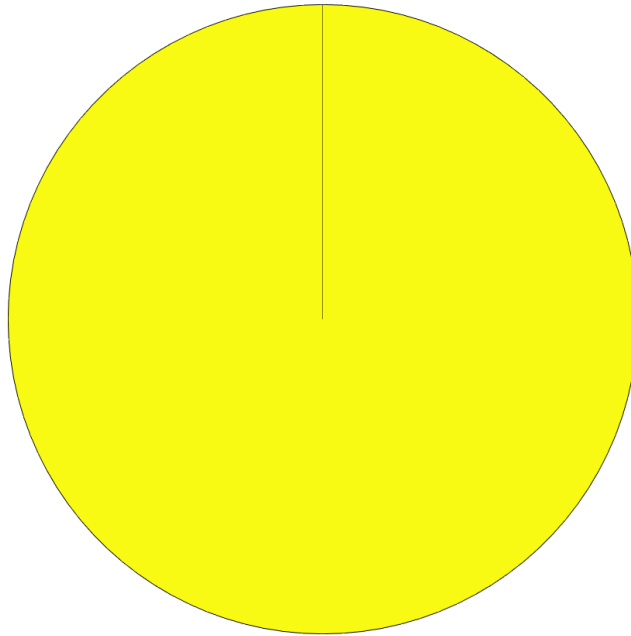


Quantitatively assigned directionality

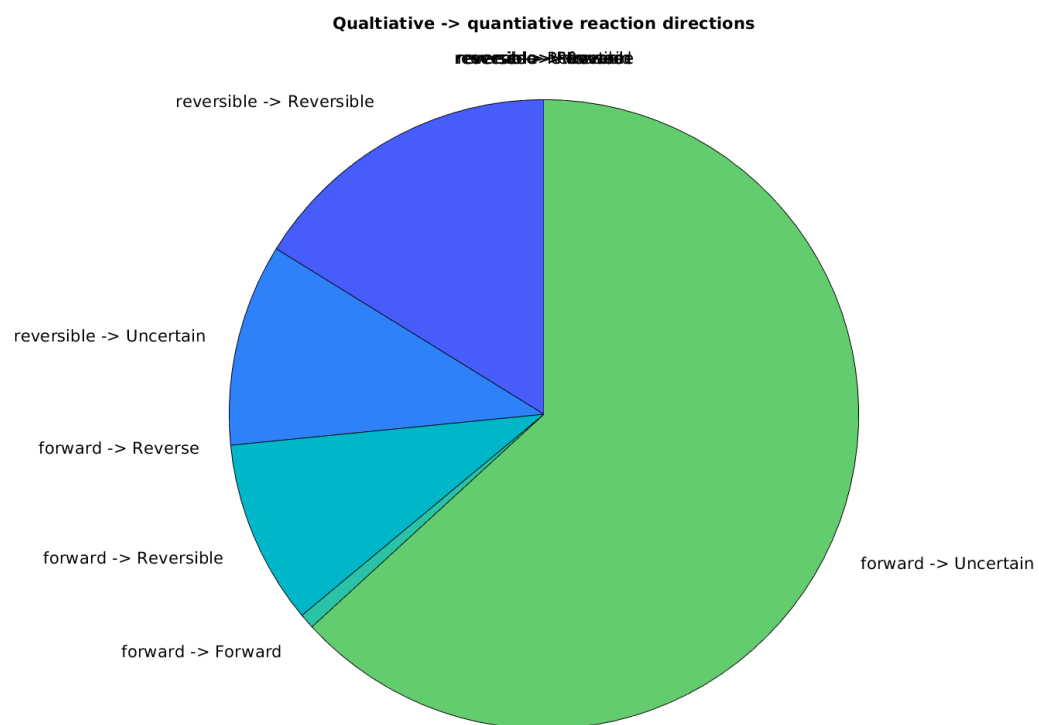


Qualitative -> quantitative changed reaction directions
(8 % of all reactions)

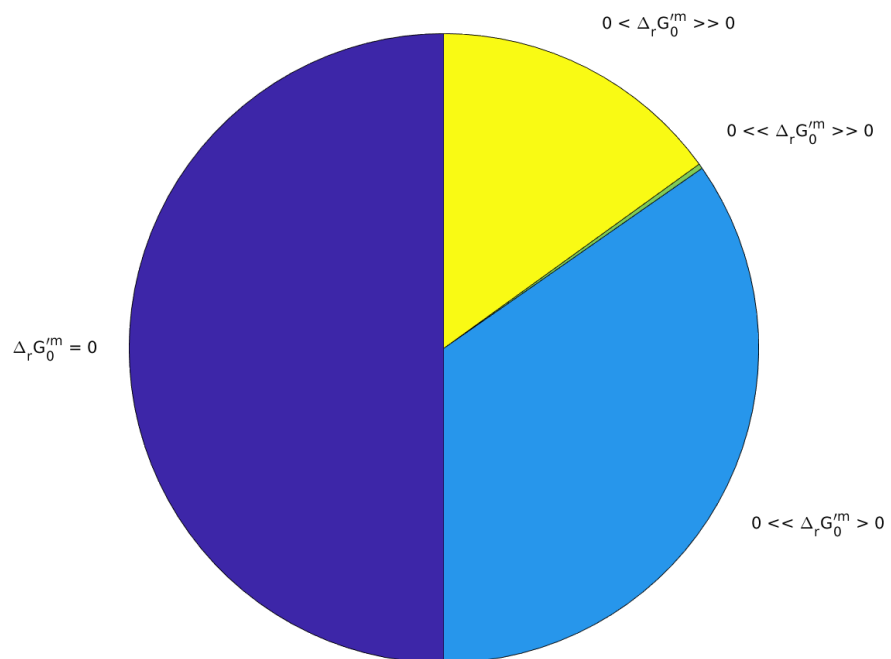
Reversible -> Reversible

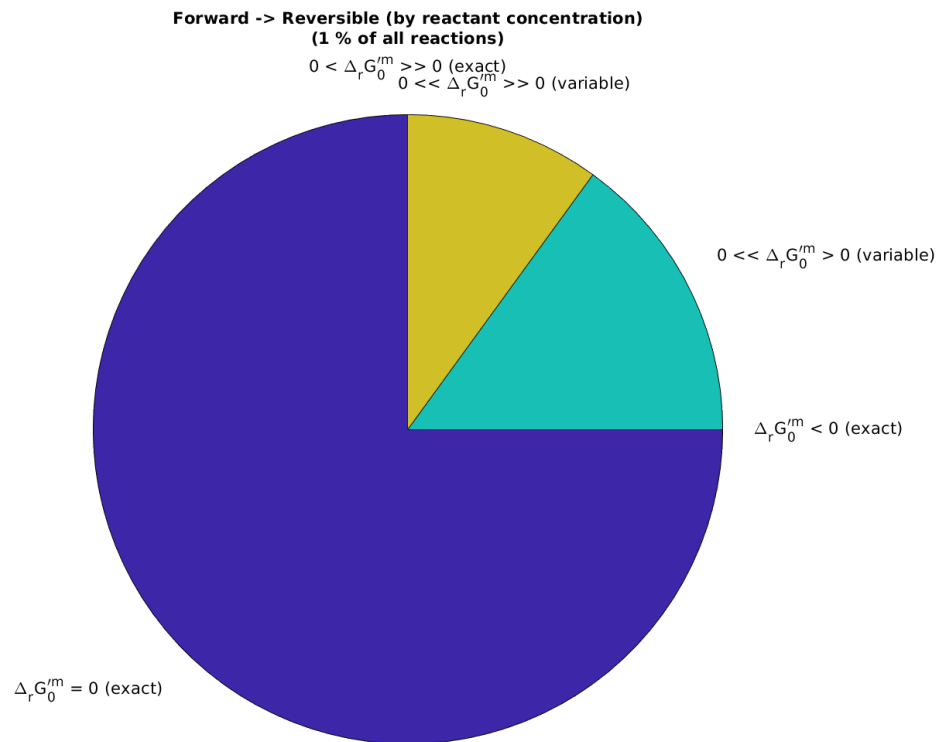


Forward -> Reversible



Forward -> Reversible (by $\Delta_r G_0^m$)
(16 % of all reactions)



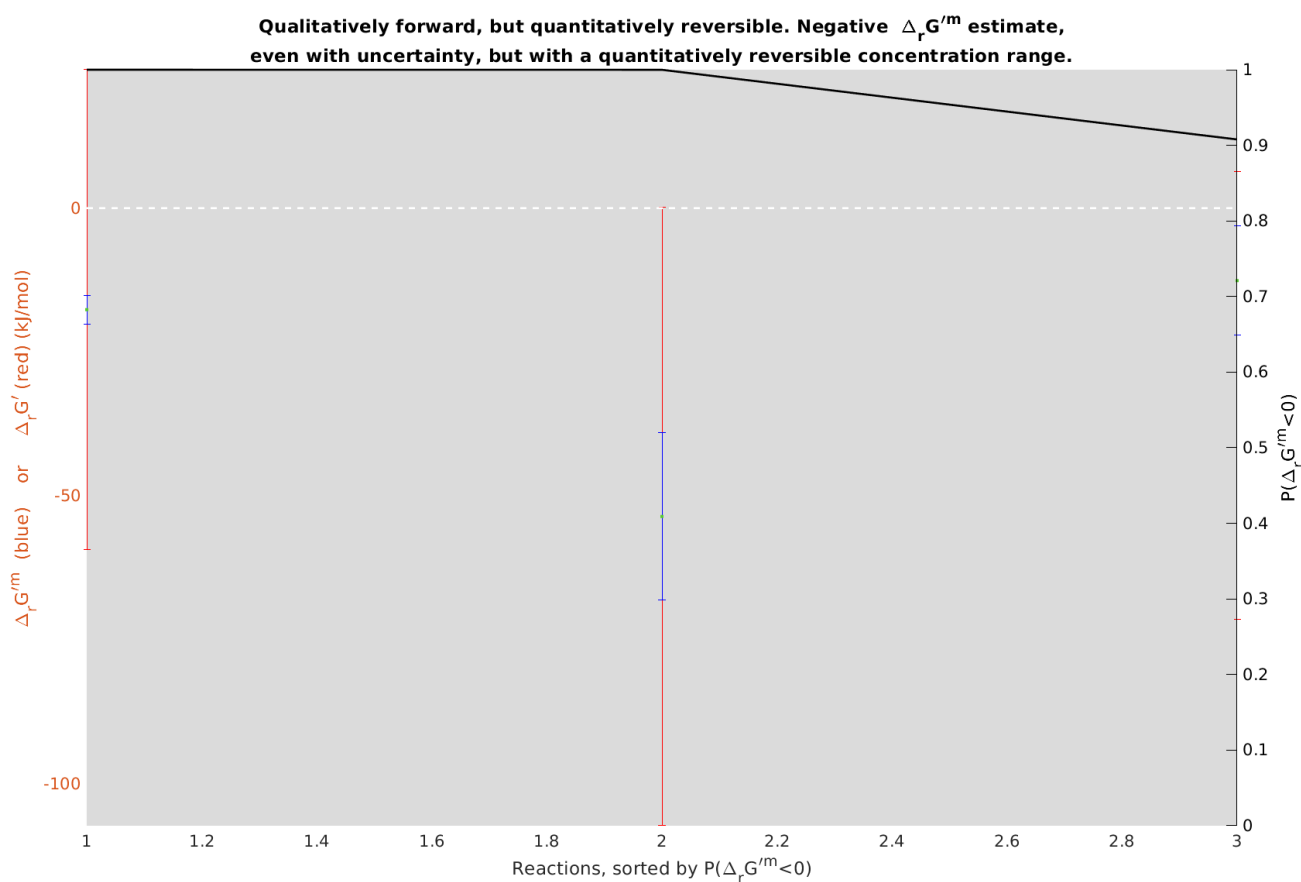
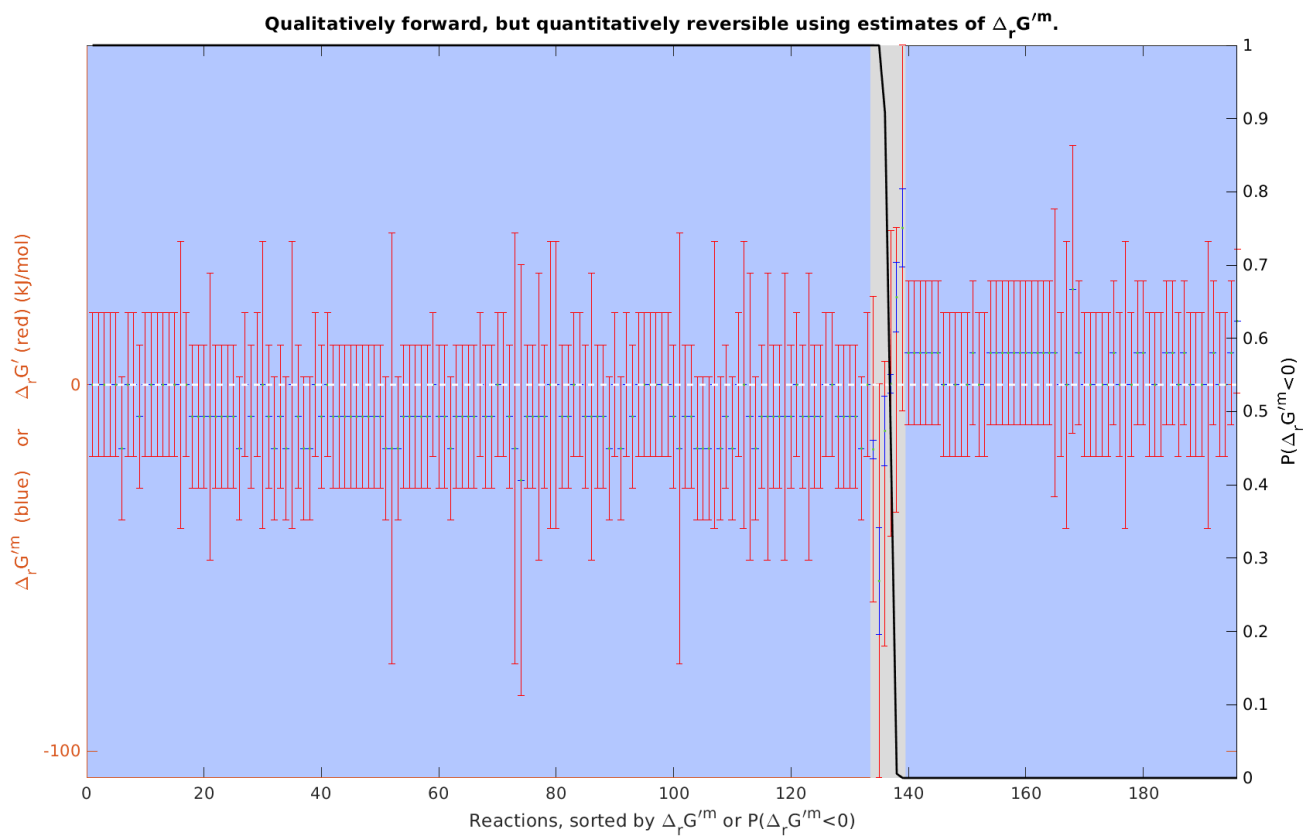


Generate figures to interpret the overall reasons for reaction directionality changes for the qualitatively forward now quantitatively reversible reactions

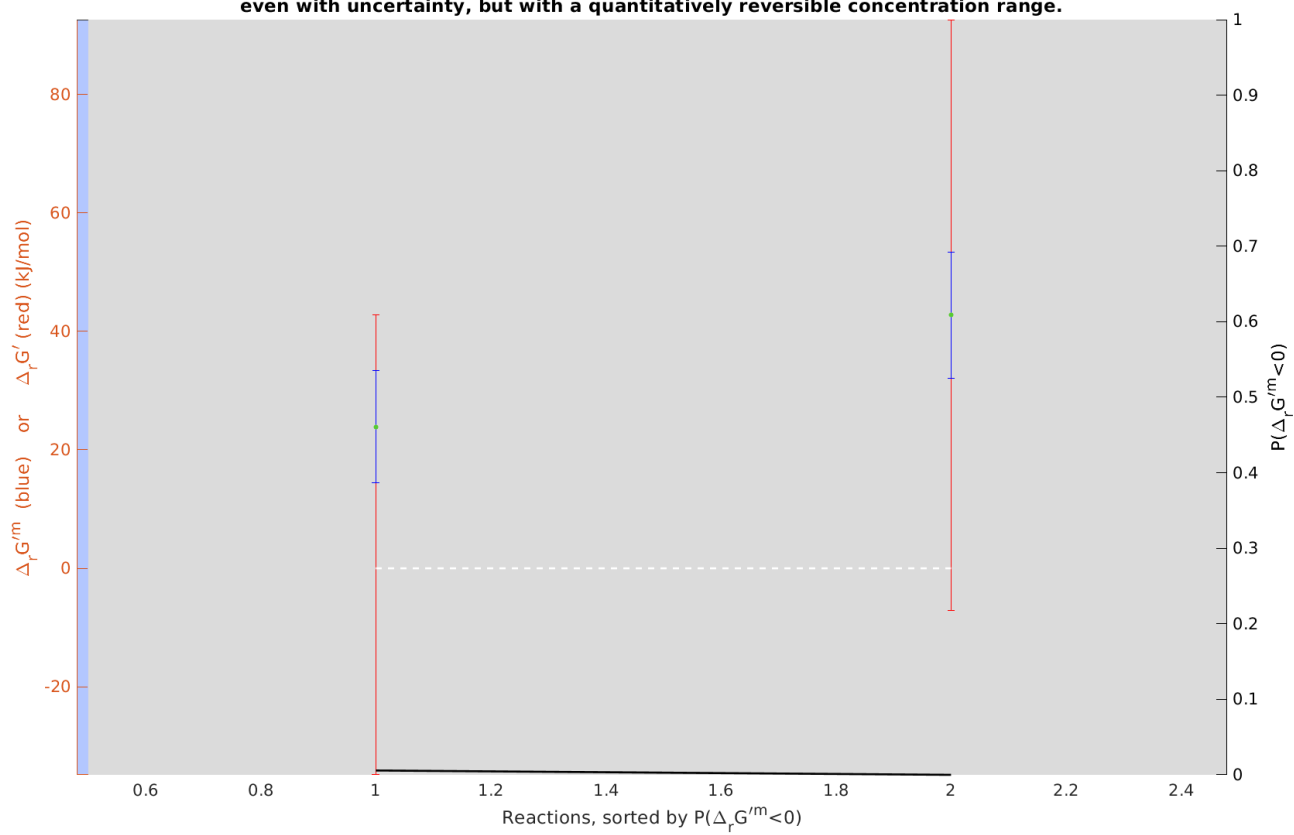
```

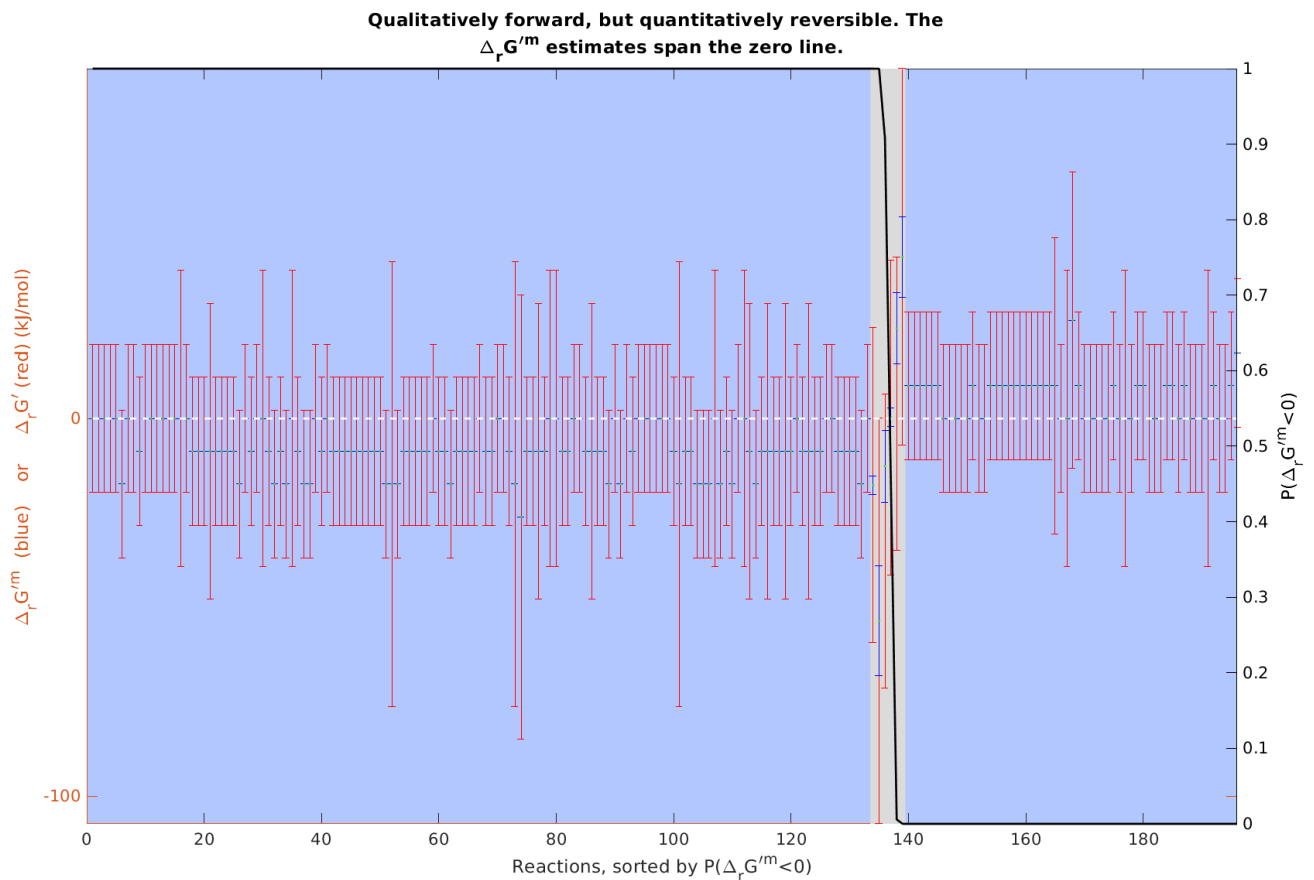
if any(directions.forward2Reversible)
    fprintf('%s\n', 'forwardReversibleFigures...');
    forwardReversibleFigures(modelThermo, directions, confidenceLevel)
end
  
```

forwardReversibleFigures...



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





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).