Thermodynamically constrain a metabolic model

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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

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. 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-  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
modelFileName = [modelName '.mat']
```

```
modelFileName =
'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
```

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
```

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,concMinDefaul
t,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,mo
del.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,mis
singFormulaeBool,balancedMetBool]...
        =
checkMassChargeBalance(model,printLevelcheckMassChargeBalance,resultsBaseFile
Name);
    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_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 n
```

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]
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,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.

Qualitiative 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, Qualitiative 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^{\primeo}<0) > 0.7
         1     of which are quantitatively reversible by range of dGt0. 0.3< P(\Delta_{r}G^{\primeo}<0) <
        59     of which are quantitatively reversible by range of dGt0. P(\Delta_{r}G^{\primeo}<0) < 0.3
        15     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
     3     of which are quantitatively reverse by dGr0t, but reversible by concentration (uncertain ne
     2     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...
```

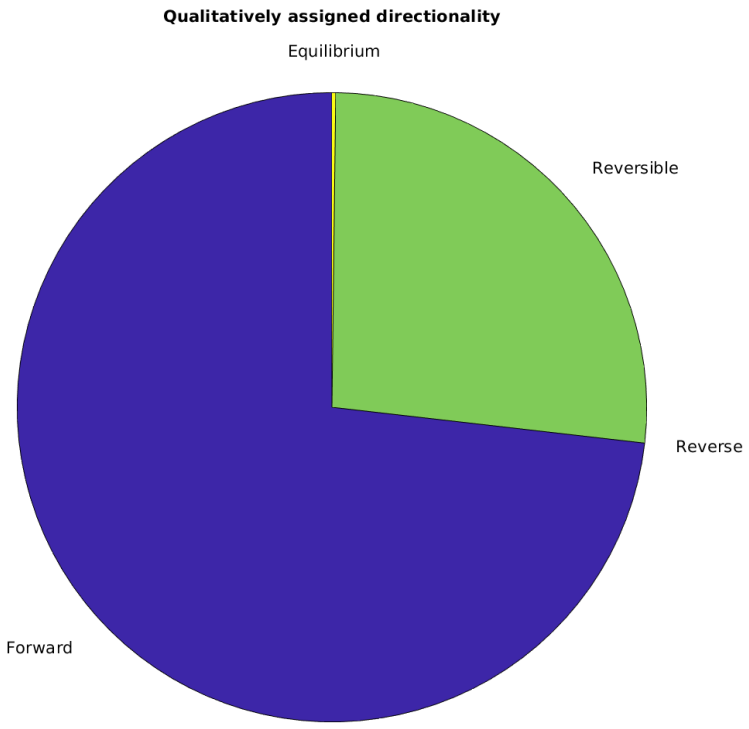
```
directionalityChangeReport(modelThermo,directions,cumNormProbCutoff,printLeve
l,resultsBaseFileName)
```

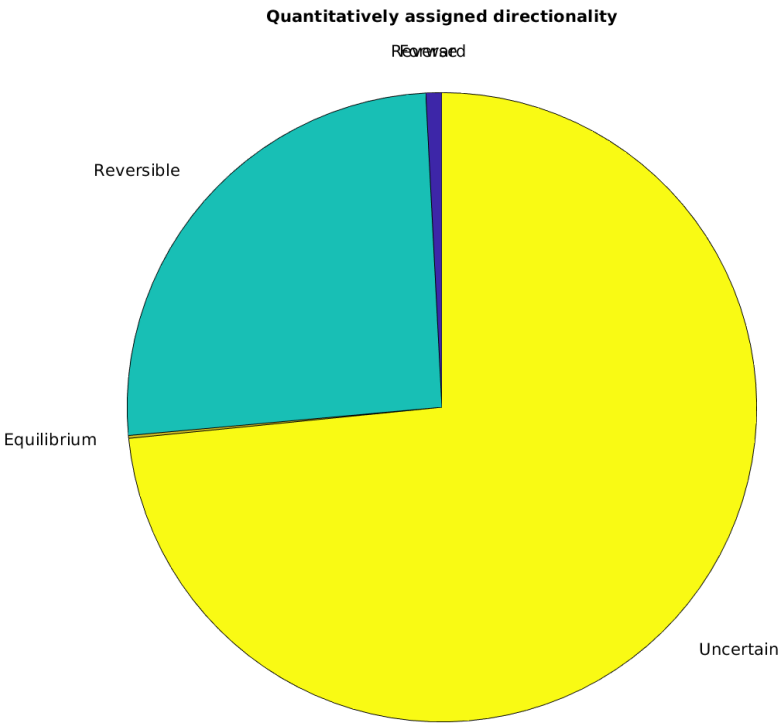
Generate pie charts with proportions of reaction directionalities and changes in directionality

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

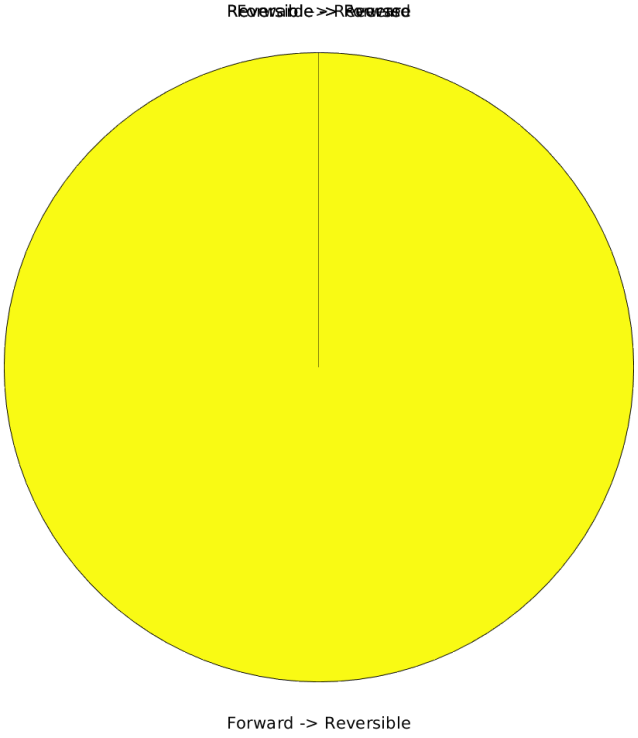
```
directionalityStatFigures...
```

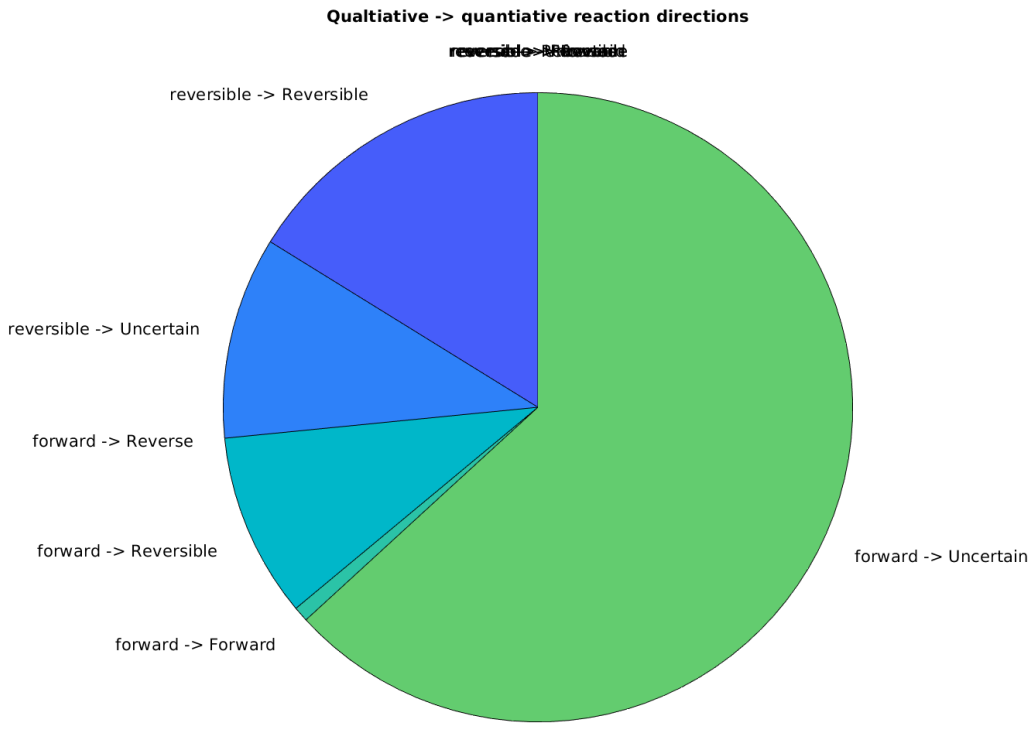
```
directionalityStatsFigures(directions,resultsBaseFileName)
```

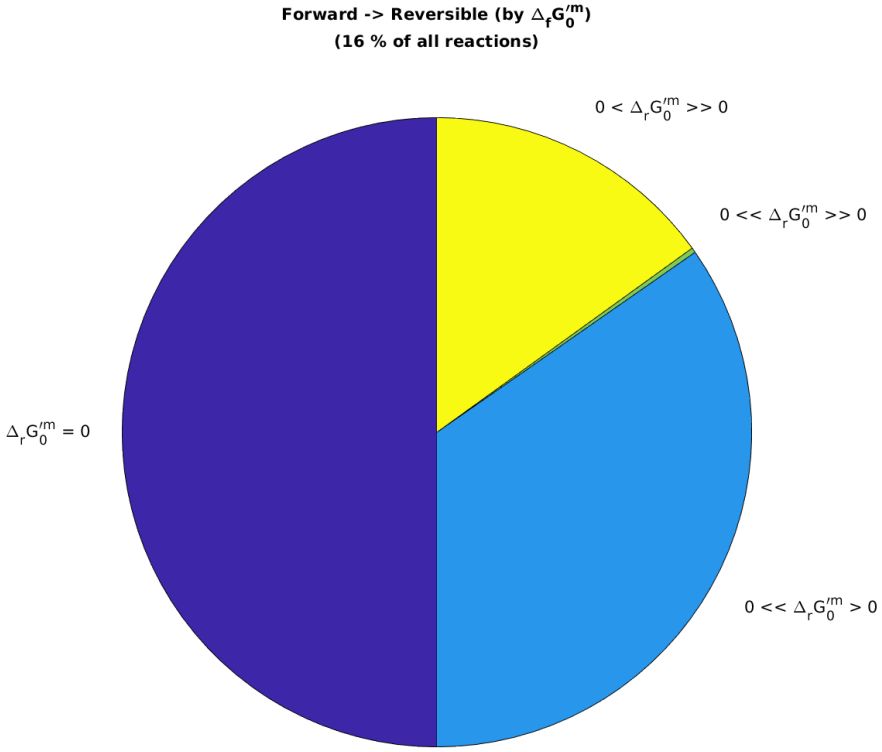

Qualitatively assigned directionality
Equilibrium
Reversible
Reverse
Forward

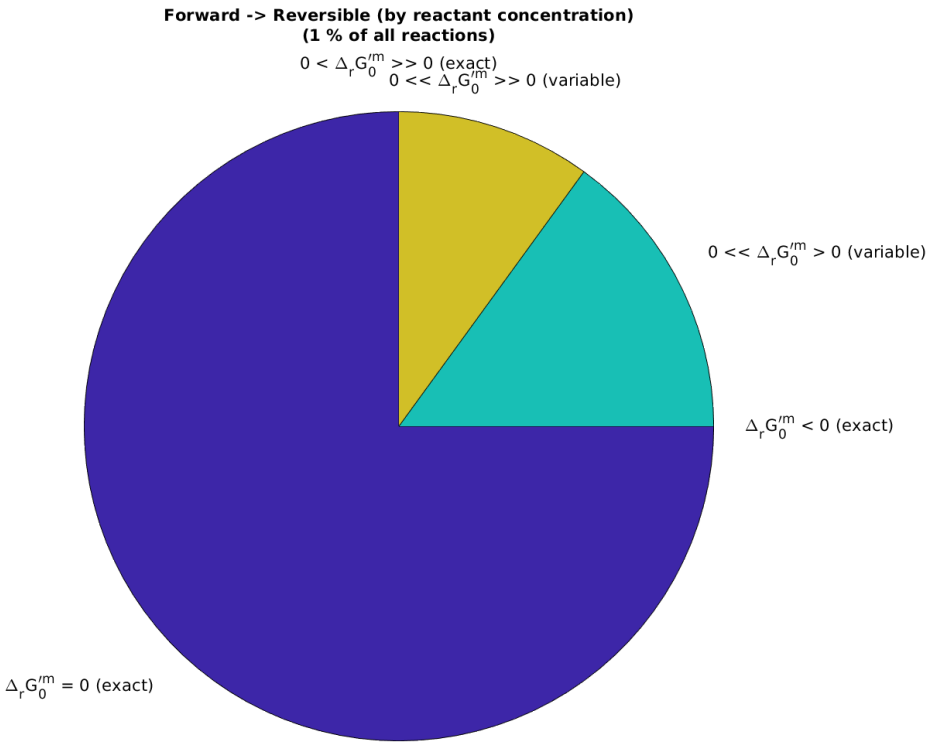

Quantitatively assigned directionality
Reversed
Reversible
Equilibrium
Uncertain

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




Qualtiative -> quantiative reaction directions
reversible -> Reversible
reversible -> Uncertain
forward -> Reverse
forward -> Reversible
forward -> Forward
forward -> Uncertain

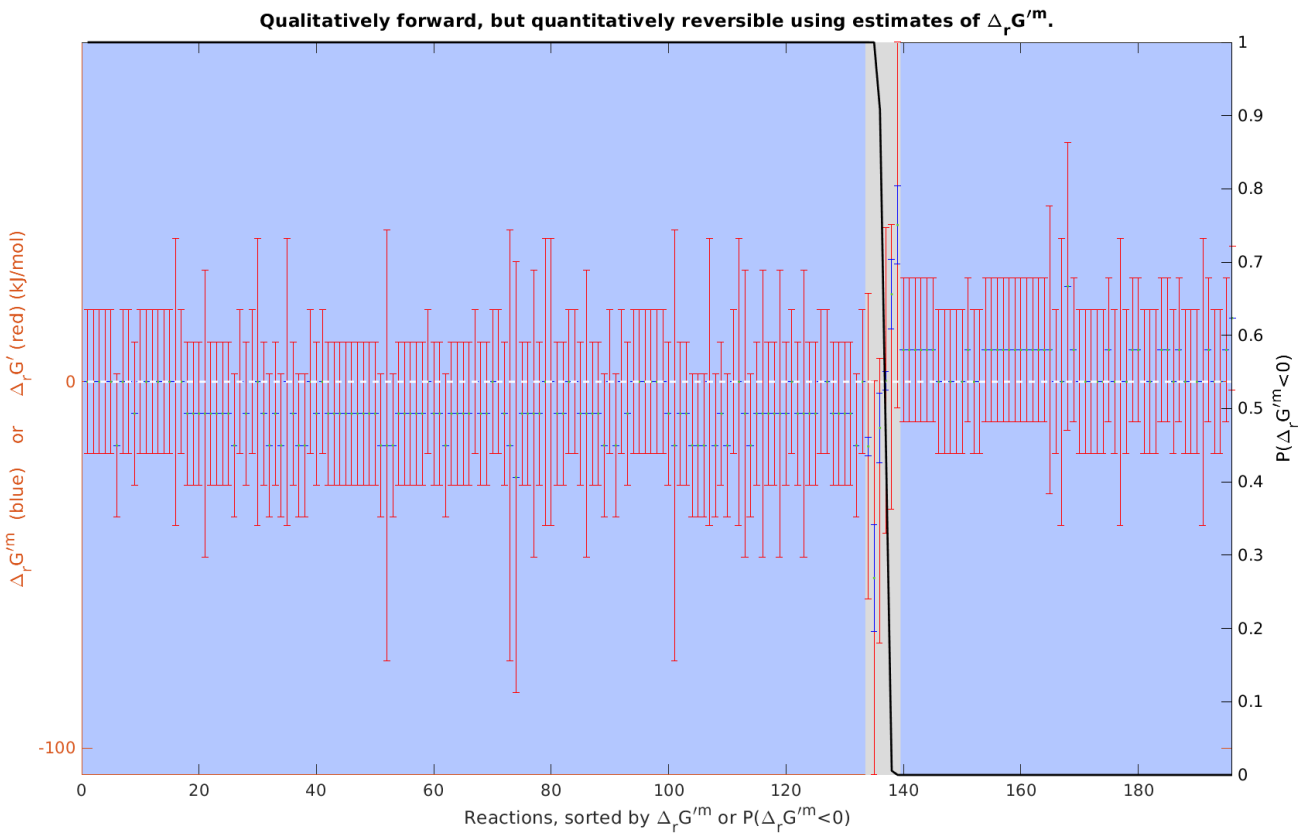

Forward -> Reversible (by $\Delta_f G_0'^m$)
(16 % of all reactions)
$0 < \Delta_r G_0'^m >> 0$
$0 << \Delta_r G_0'^m >> 0$
$\Delta_r G_0'^m = 0$
$0 << \Delta_r G_0'^m > 0$

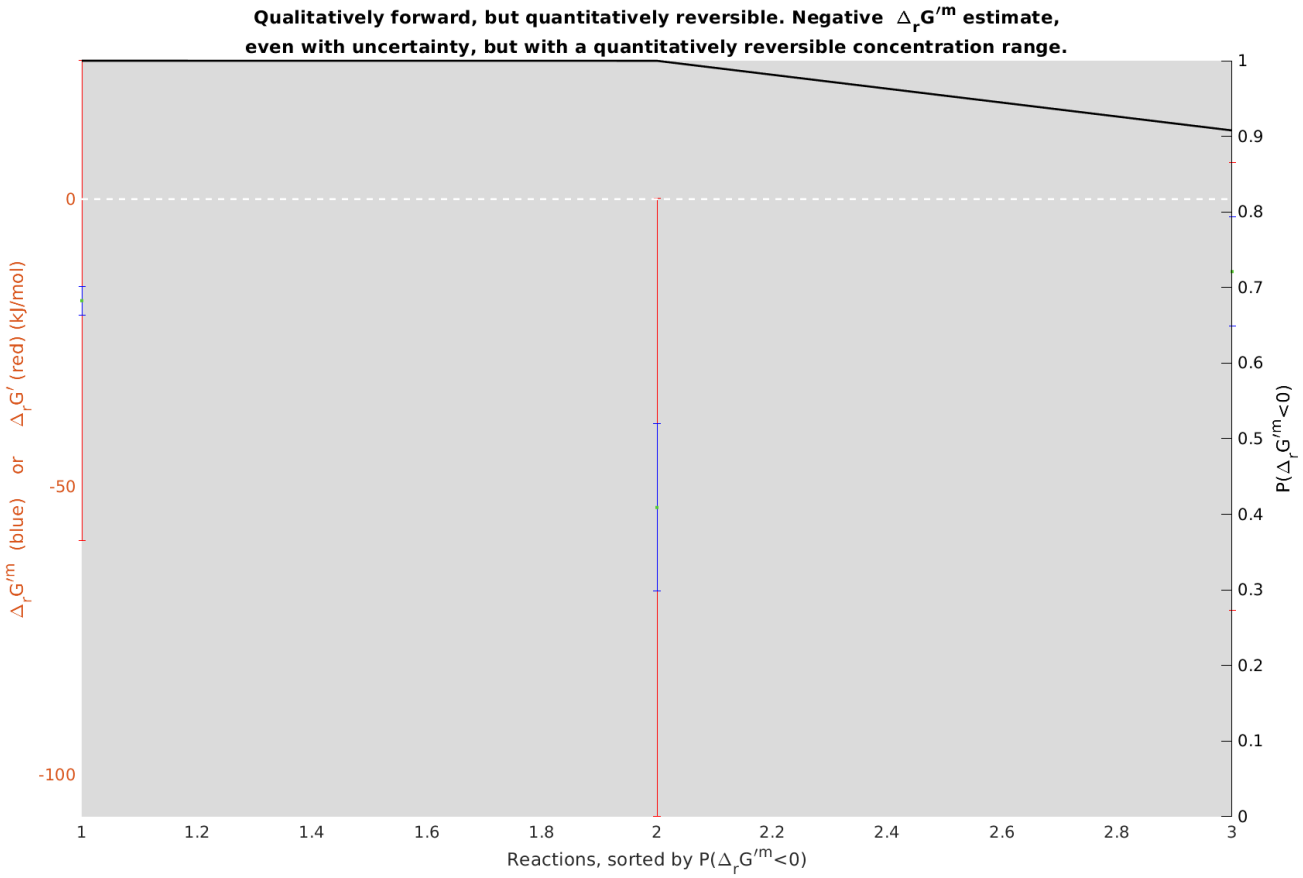


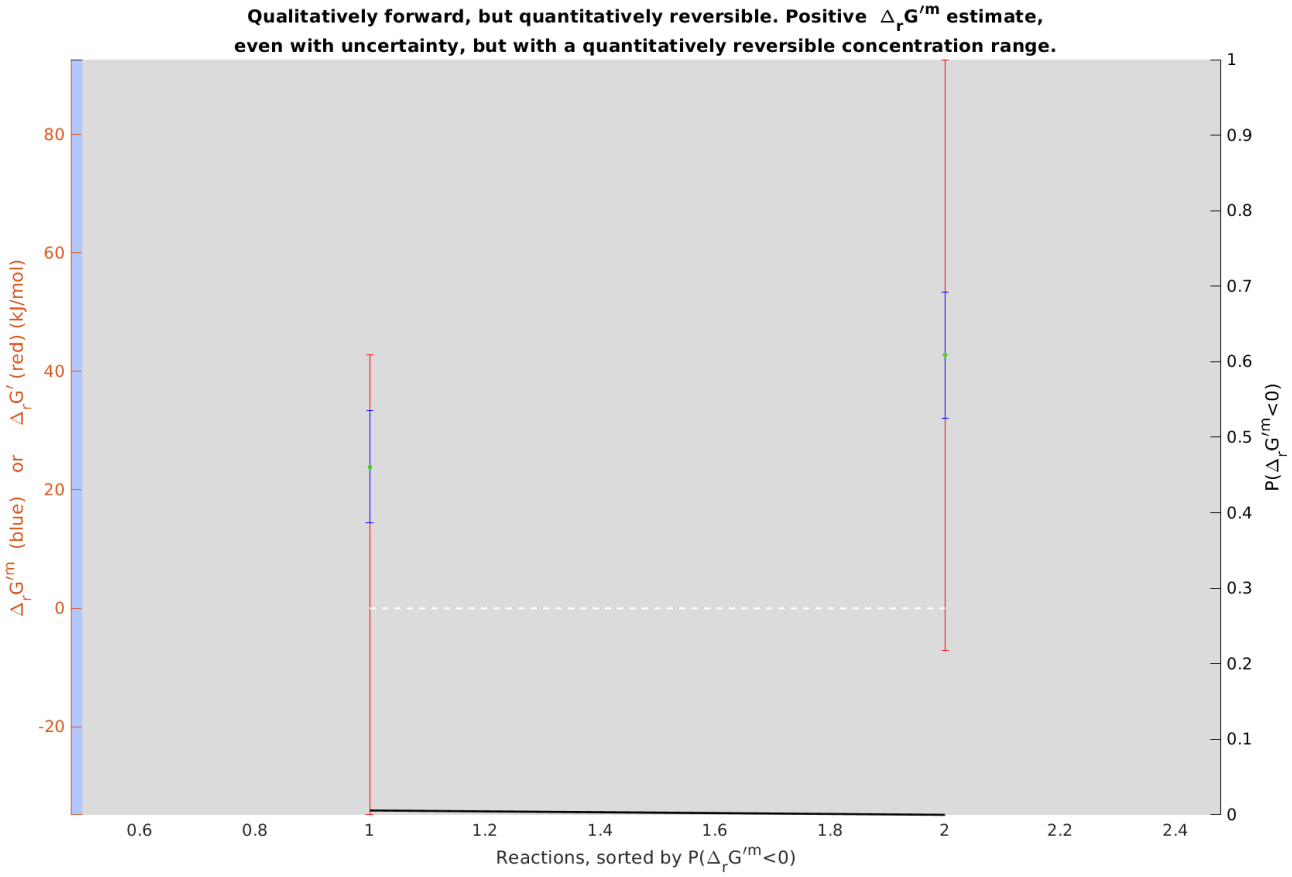
Generate figures to interpret the overall reasons for reaction directionality changes for the qualitatively forward now quantiatiavely reversible reactions

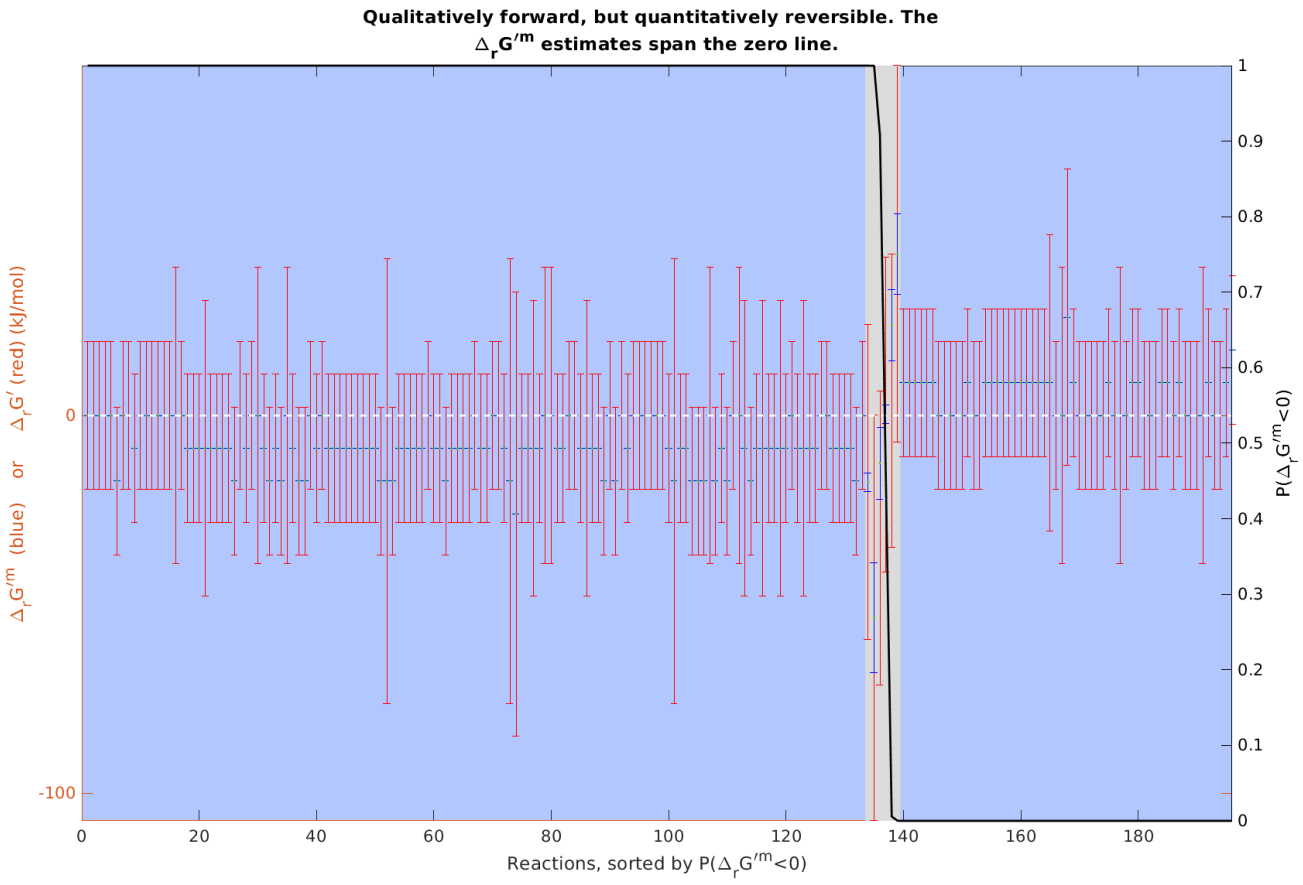
```
if any(directions.forward2Reversible)
    fprintf('%s\n','forwardReversibleFigures...');
    forwardReversibleFigures(modelThermo,directions,confidenceLevel)
end
```

```
forwardReversibleFigures...
```


Qualitatively forward, but quantitatively reversible using estimates of $\Delta_r G'^m$.
$\Delta_r G'^m$ (blue) or $\Delta_r G'$ (red) (kJ/mol)
$P(\Delta_r G'^m<0)$
Reactions, sorted by $\Delta_r G'^m$ or $P(\Delta_r G'^m<0)$


Qualitatively forward, but quantitatively reversible. Negative $\Delta_r G'^m$ estimate,
even with uncertainty, but with a quantitatively reversible concentration range.
$\Delta_r G'^m$ (blue) or $\Delta_r G'$ (red) (kJ/mol)
$P(\Delta_r G'^m<0)$
Reactions, sorted by $P(\Delta_r G'^m<0)$


Qualitatively forward, but quantitatively reversible. Positive $\Delta_r G'^m$ estimate,
even with uncertainty, but with a quantitatively reversible concentration range.
$\Delta_r G'^m$ (blue) or $\Delta_r G'$ (red) (kJ/mol)
$P(\Delta_r G'^m<0)$
Reactions, sorted by $P(\Delta_r G'^m<0)$
80
60
40
20
0
-20
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
2.4



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).

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