Component contribution: analysis of updated method applied to Recon3D

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Reviewers:

Table of Contents

Author: Ronan Fleming, School of Medicine, University of Galway....1
Reviewers: 1
INTRODUCTION....1
PROCEDURE....1
Configure the environment....1
Component Contribution....2
Comparison of weighting of reactant and group contribution for training metabolites....2
Analyse reactant contribution....7
Analyse group contribution....10
Analyse component contribution....16
Comparison of weighting of reactant and group contribution for test metabolites....22
Analyse reacting moieties in the test model....27
Reaction Component contribution taking into account reacting moieties only....33
Estimated standard reaction Gibbs energy....33
Estimated standard metabolite Gibbs energy vs molecular mass....35
Estimated standard reaction Gibbs energy vs bonds broken and formed....36

INTRODUCTION

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.

```
aPath = which('initVonBertalanffy');
basePath = strrep(aPath,'vonBertalanffy/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/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
```

```
initVonBertalanffy
```

```
ChemAxon Marvin Beans is installed and working.
    linux-vdso.so.1 (0x00007ffc78f8b000)
    libc.so.6 => /lib/x86_64-linux-gnu/libc.so.6 (0x00007fd52933b000)
    libopenbabel.so.5 => /usr/lib/libopenbabel.so.5 (0x00007fd5290eb000)
    libstdc++.so.6 => /usr/lib/x86_64-linux-gnu/libstdc++.so.6 (0x00007fd528ed1000)
    libgcc_s.so.1 => /usr/local/bin/MATLAB/R2021a/sys/os/glnxa64/libgcc_s.so.1 (0x00007fd528cb9000)
    /lib64/ld-linux-x86-64.so.2 (0x00007fd52955b000)
    libdl.so.2 => /lib/x86_64-linux-gnu/libdl.so.2 (0x00007fd528cb1000)
    libz.so.1 => /lib/x86_64-linux-gnu/libz.so.1 (0x00007fd528c95000)
    libm.so.6 => /lib/x86_64-linux-gnu/libm.so.6 (0x00007fd528b46000)
    libgomp.so.1 => /usr/lib/x86_64-linux-gnu/libgomp.so.1 (0x00007fd528b01000)
    libpthread.so.0 => /lib/x86_64-linux-gnu/libpthread.so.0 (0x00007fd528ade000)

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
```

Load data input for component contribution method

```
load('data_prior_to_componentContribution')
```

Component Contribution

Run component contribution method

```
param.debug = 1;
[model,solution] = componentContribution(model,combinedModel,param);
```

```
Running Component Contribution method
```

Comparison of weighting of reactant and group contribution for training metabolites

```
X = combinedModel.S;
```

```
XR = solution.PR_St*X;
XN = solution.PN_St*X;
XNR = (solution.PN_St - solution.PN_StGGt)*X;
XNN = solution.PN_StGGt*X;
```

Check that the decomposition into different components is complete

```
norm(X - (XR + XN),'inf')
```

```
ans = 4.7851e-14
```

```
norm(XN - (XNR + XNN),'inf')
```

```
ans = 9.2198e-14
```

```
norm(X - (XR + XNR + XNN),'inf')
```

```
ans = 8.1953e-14
```

Stoichiometric degree

```
dX = diag(X*X');
fprintf('%u%s\n',nnz(dX),' metabolites with non-zero training stoichiometric
degree')
```

```
668 metabolites with non-zero training stoichiometric degree
```

```
fprintf('%u%s\n',nnz(dX==0),' metabolites with zero training stoichiometric
degree')
```

```
2998 metabolites with zero training stoichiometric degree
```

```
dXR = diag(XR*XR');
fprintf('%u%s\n',nnz(dXR),' metabolites with non-zero training
stoichiometric degree, in the range of S''')
```

```
671 metabolites with non-zero training stoichiometric degree, in the range of S'
```

```
fprintf('%u%s\n',nnz(dXR==0),' metabolites with zero training stoichiometric
degree, in the range of S''')
```

```
2995 metabolites with zero training stoichiometric degree, in the range of S'
```

```
dXN = diag(XN*XN');
fprintf('%u%s\n',nnz(dXN),' metabolites with non-zero training
stoichiometric degree, in the nullspace of S''')
```

```
654 metabolites with non-zero training stoichiometric degree, in the nullspace of S'
```

```
fprintf('%u%s\n',nnz(dXN==0),' metabolites with zero training stoichiometric
degree, in the nullspace of S''')
```

```
3012 metabolites with zero training stoichiometric degree, in the nullspace of S'
```

```
dXNR = diag(XNR*XNR');
```

```
fprintf('%u%s\n',nnz(dXNR),' metabolites with non-zero training
stoichiometric degree, in the nullspace of S'' and G''x in the range of
G''S')
```

```
2656 metabolites with non-zero training stoichiometric degree, in the nullspace of S' and G'x in the range
```

```
fprintf('%u%s\n',nnz(dXNR==0),' metabolites with zero training
stoichiometric degree, in the nullspace of S'' and G''x in the range of
G''S')
```

```
1010 metabolites with zero training stoichiometric degree, in the nullspace of S' and G'x in the range of
```

```
dXNN = diag(XNN*XNN');
fprintf('%u%s\n',nnz(dXNN),' metabolites with non-zero training
stoichiometric degree, in the nullspace of S'' and in the nullspace of
S''GG'' ')
```

```
2648 metabolites with non-zero training stoichiometric degree, in the nullspace of S' and in the nullspace
```

```
fprintf('%u%s\n',nnz(dXNN==0),' metabolites with zero training
stoichiometric degree, in the nullspace of S'' and in the nullspace of
S''GG'' ')
```

```
1018 metabolites with zero training stoichiometric degree, in the nullspace of S' and in the nullspace of
```

```
norm(dX - (dXR + dXN),'inf')
```

```
ans = 6.5938e-12
```

```
norm(dXN - (dXNR + dXNN),'inf')
```

```
ans = 3.2797e+03
```

```
norm(dX - (dXR + dXNR + dXNN),'inf')
```

```
ans = 3.2797e+03
```

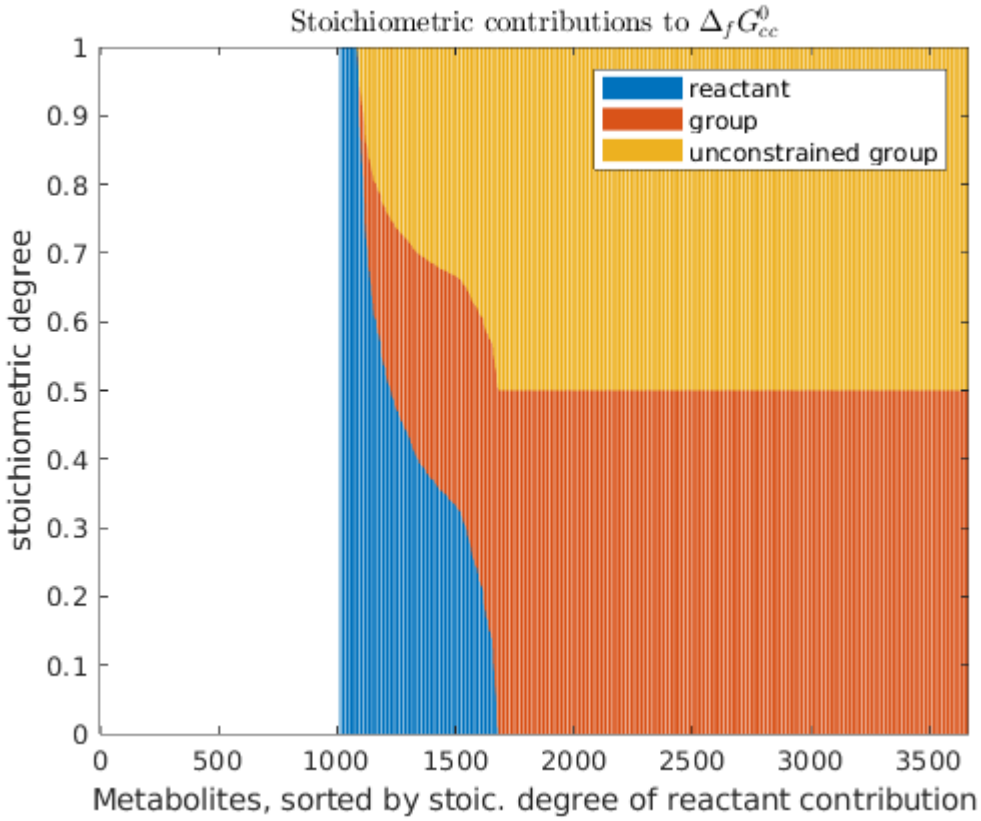
```
norm(dX.^2 - (dXR.^2 + dXNR.^2 + dXNN.^2),'inf')
```

```
ans = 5.3781e+06
```

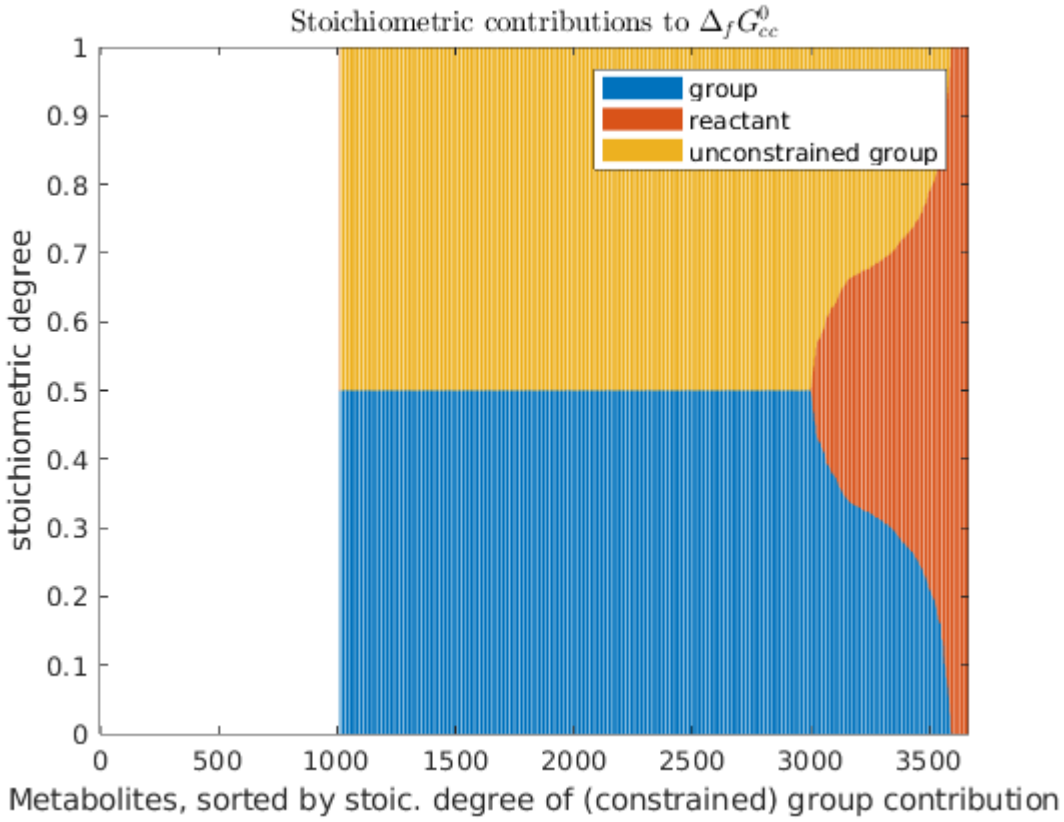
Sort by stoichiometric degree of reactant contribution (for each metabolite)

```
dXtotal = dXR + dXNR + dXNN;
Y = [dXR./dXtotal,dXNR./dXtotal,dXNN./dXtotal];
[dXRsorted,xi]=sort(dXR./dXtotal,'descend');
figure
bar(Y(xi,:),'stacked')
ylim([0 1])
title('Stoichiometric contributions to $\Delta_{f} G^{0}_{cc}
$','Interpreter','latex')
xlabel('Metabolites, sorted by stoic. degree of reactant contribution')
```

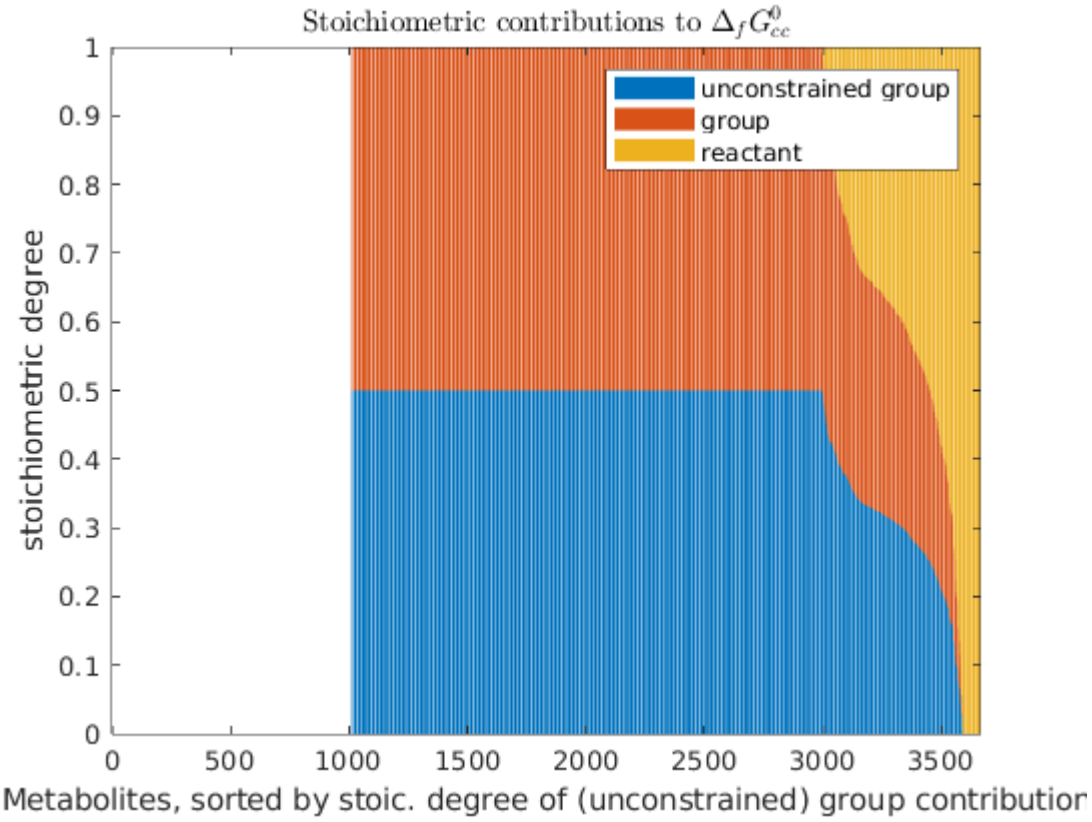
```
ylabel('stoichiometric degree')
legend({'reactant','group','unconstrained group'})
```




```
Y = [dXNR./dXtotal,dXR./dXtotal,dXNN./dXtotal];
[~,xi]=sort(dXNR./dXtotal,'descend');
figure
bar(Y(xi,:),'stacked')
ylim([0 1])
title('Stoichiometric contributions to $\Delta_{f} G^{0}_{cc}
$','Interpreter','latex')
xlabel('Metabolites, sorted by stoic. degree of (constrained) group
contribution')
ylabel('stoichiometric degree')
legend({'group','reactant','unconstrained group'})
```

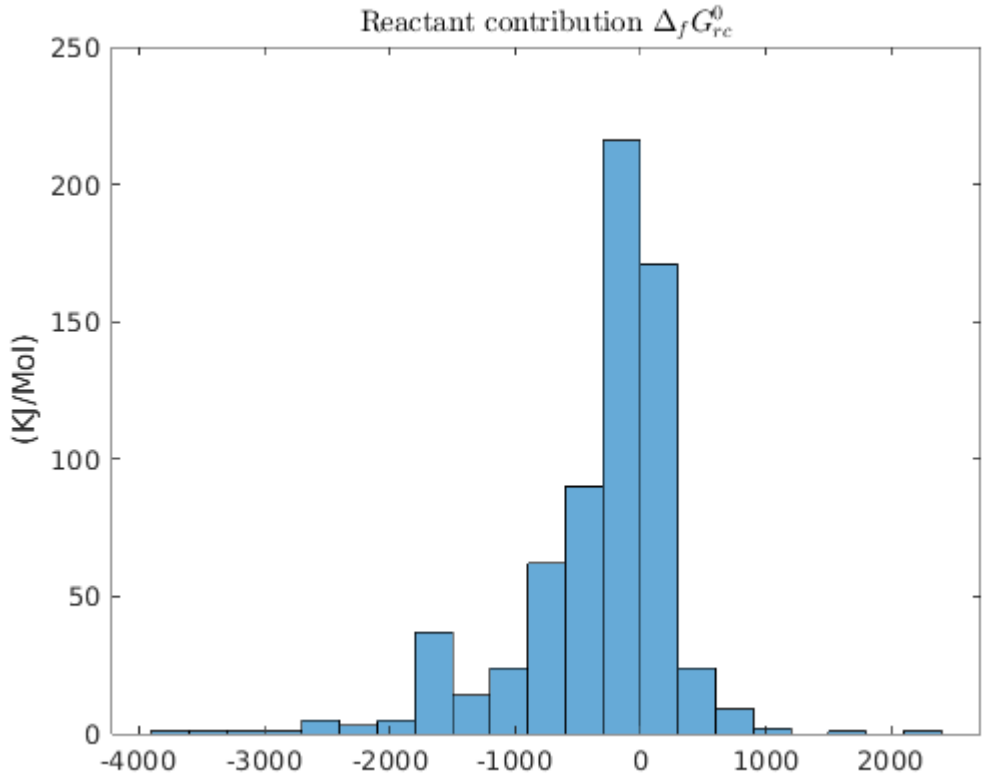



```
Y = [dXNN./dXtotal,dXNR./dXtotal,dXR./dXtotal];
[~,xi]=sort(dXNN./dXtotal,'descend');
figure
bar(Y(xi,:),'stacked')
ylim([0 1])
title('Stoichiometric contributions to $\Delta_{f} G^{0}_{cc}
$','Interpreter','latex')
xlabel('Metabolites, sorted by stoic. degree of (unconstrained) group
contribution')
ylabel('stoichiometric degree')
legend({'unconstrained group','group','reactant'})
```



Analyse reactant contribution

```
figure
histogram(solution.DfG0_rc(~solution.unconstrainedDfG0_rc))
title('$\textrm{Reactant contribution } \Delta_{f} G^{0}_{rc}
$','Interpreter','latex')
ylabel('(KJ/Mol)')
```




```
nCombinedMet=size(combinedModel.S,1);
fprintf('%u%s\n',nCombinedMet,' formation energies')
```

```
3666 formation energies
```

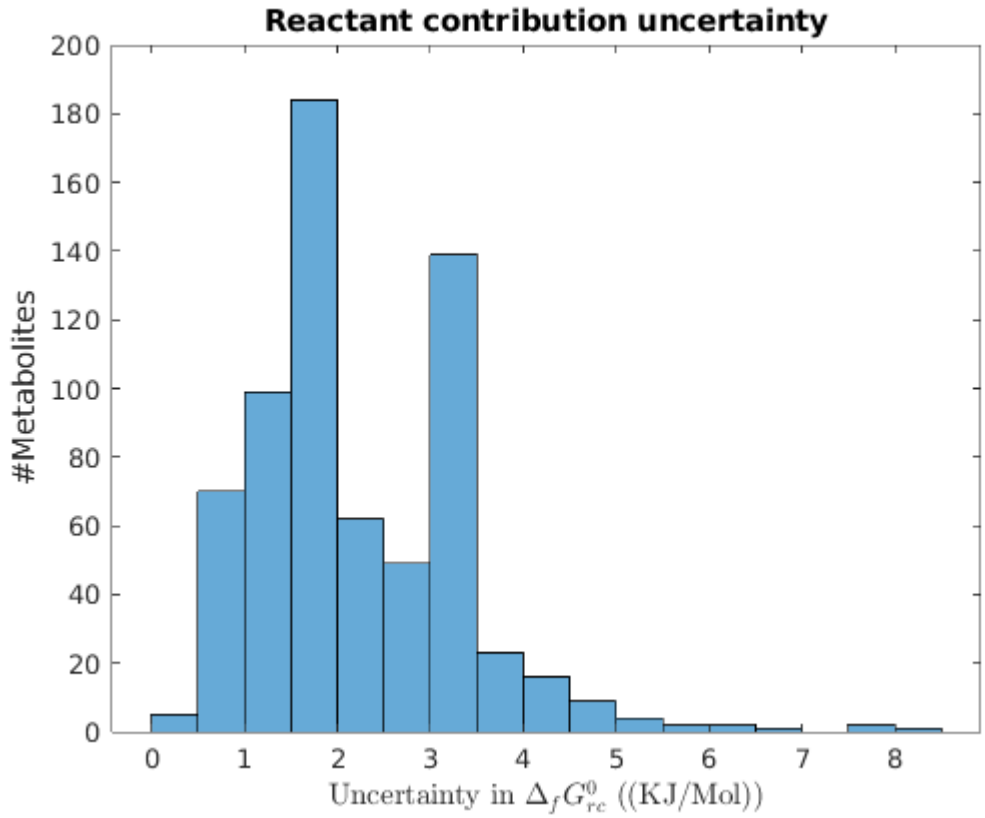
```
fprintf('%u%s\n',nnz(solution.unconstrainedDfG0_rc),' of which DfG0_rc are
unconstrained. i.e., number of formation energies that cannot be determined
by reactant contribution')
```

```
2998 of which DfG0_rc are unconstrained. i.e., number of formation energies that cannot be determined by r
```

```
fprintf('%g%s\n',nnz(solution.unconstrainedDfG0_rc)/nCombinedMet,' =
fraction of DfG0 unconstrained by reactant contribution.')
```

```
0.817785 = fraction of DfG0 unconstrained by reaction contribution.
```

```
figure
histogram(solution.DfG0_rc_Uncertainty(~solution.unconstrainedDfG0_rc))
title('Reactant contribution uncertainty')
ylabel('#Metabolites')
xlabel('Uncertainty in $\Delta_{f} G^{0}_{rc}$  ((KJ/
Mol))','Interpreter','latex')
```



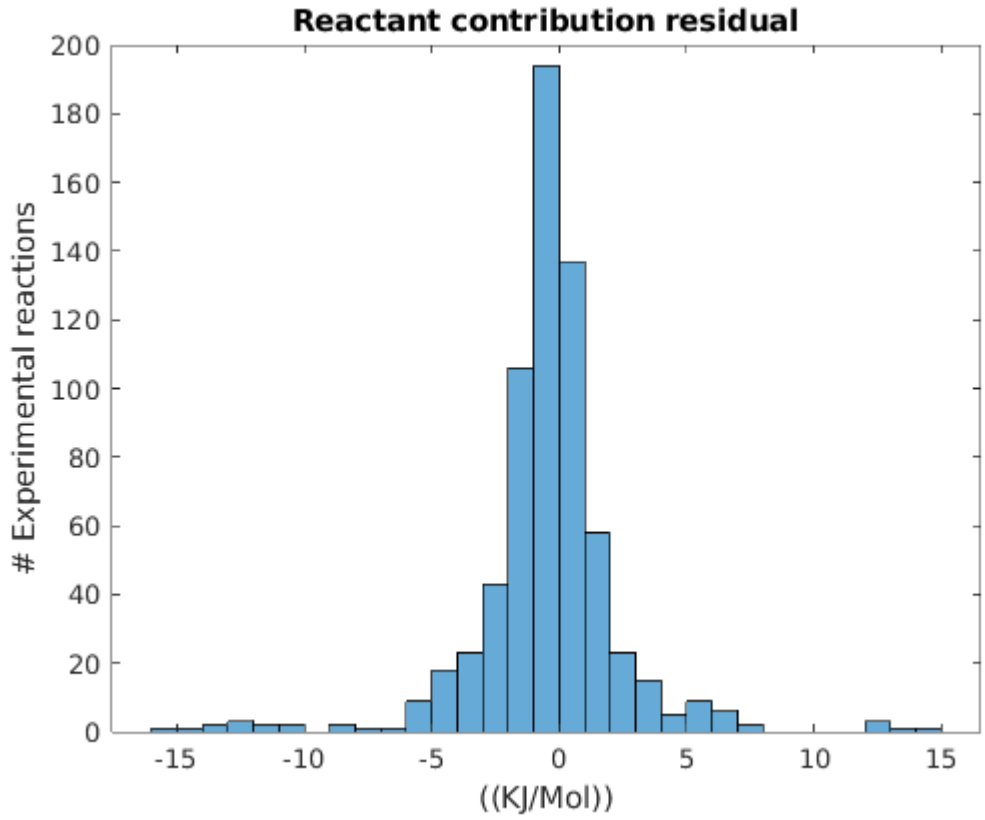

```
fprintf('%g%s\n',nnz(solution.DfG0_rc_Uncertainty==0 &
~solution.unconstrainedDfG0_rc),' number of zero uncertainty in constrained
DfG0_rc')
```

```
0 number of zero uncertainty in constrained DfG0_rc
```

```
fprintf('%g%s\n',nnz(solution.DfG0_rc_Uncertainty==0 &
solution.unconstrainedDfG0_rc),' number of zero uncertainty in unconstrained
DfG0_rc')
```

```
2998 number of zero uncertainty in unconstrained DfG0_rc
```

```
figure;
histogram(solution.e_rc(~solution.unconstrainedDfG0_rc))
text(-30,700,{['MSE = ' num2str(solution.MSE_rc)],['MAE = '
num2str(solution.MAE_rc)]});
title('Reactant contribution residual')
xlabel('((KJ/Mol))');
ylabel('# Experimental reactions')
```



Experiments contributing the largest to residuals in the reactant contribution method

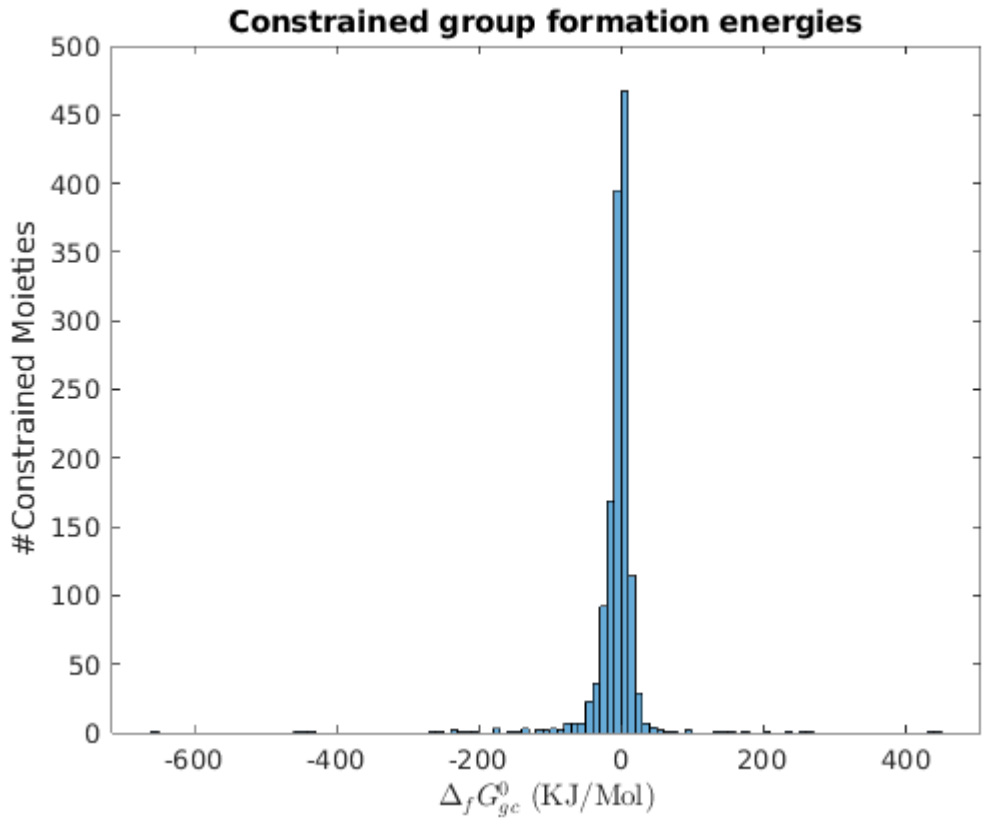
```
 [rcErrorSorted,rcSI]=sort(solution.e_rc);
 N=10;
 for i=1:N
     rxnFormula =
printRxnFormula(combinedModel,'rxnAbbrList',combinedModel.rxns(rcSI(i)),'prin
tFlag',0);

fprintf('%g\t%s\t%s\n',solution.e_rc(rcSI(i)),combinedModel.rxns{rcSI(i)},rxn
Formula{1});
 end
```

```
-31.6414    TECRDB_1919    C00002 + C01281  -> C00013 + C01299
-26.9545    TECRDB_1929    C00002 + C01281  -> C00013 + C01299
-21.9955    TECRDB_1920    C00002 + C01281  -> C00013 + C01299
-20.5893    FORM_C00093     -> C00093
-17.7694    FORM_C01127     -> C01127
-15.2367    TECRDB_539    C00002 + C00300  -> C00008 + C02305
-15.1381    TECRDB_2655    C00003 + C00197  -> C00004 + C03232
-14.375    TECRDB_3728    C00003 + C00469  -> C00004 + C00084
-14.2817    TECRDB_374    C00006 + C00342  -> C00005 + C00343
-13.4218    TECRDB_237    C00002 + C00062  -> C00008 + C05945
```

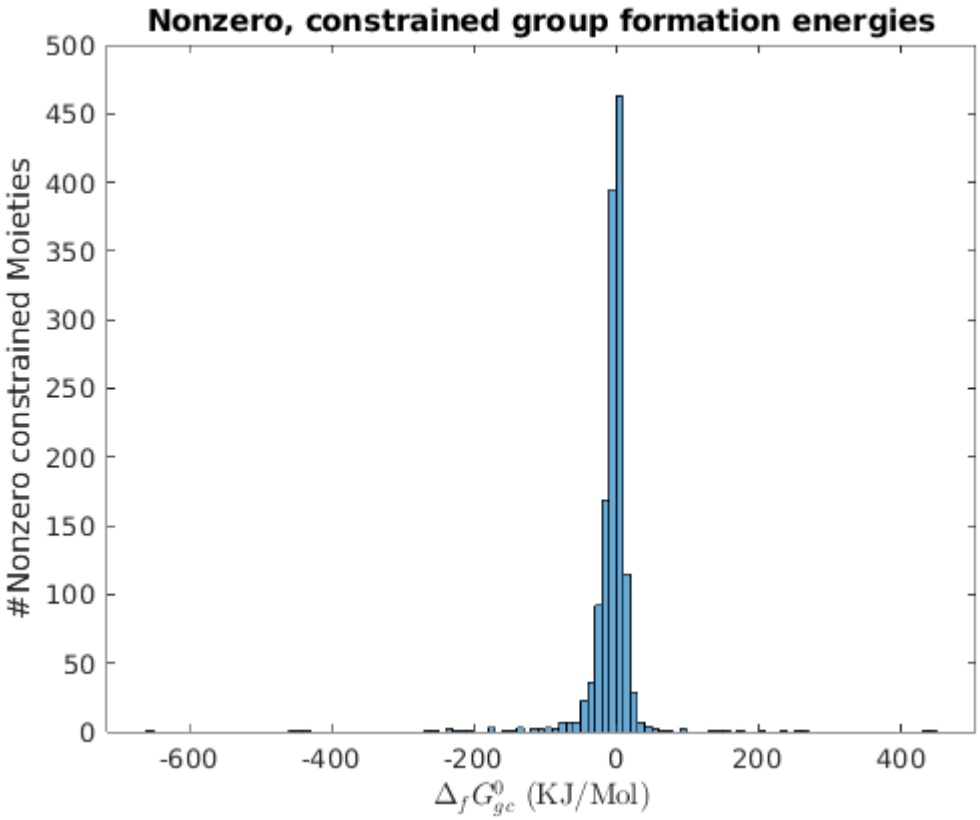
Analyse group contribution

```
 figure
```

```
 histogram(solution.DfG0_gc(~solution.unconstrainedDfG0_gc))
 title('Constrained group formation energies')
xlabel('$\Delta_{f} G^{0}_{gc}$ (KJ/Mol)','Interpreter','latex')
ylabel('#Constrained Moieties')
```




```
figure
histogram(solution.DfG0_gc(solution.DfG0_gc~=0 &
~solution.unconstrainedDfG0_gc))
title('Nonzero, constrained group formation energies')
xlabel('$\Delta_{f} G^{0}_{gc}$ (KJ/Mol)','Interpreter','latex')
ylabel('#Nonzero constrained Moieties')
```




```
nGroups =size(combinedModel.G,2);
fprintf('%u%s\n',nGroups,' estimated group formation energies')
```

```
5189 estimated group formation energies
```

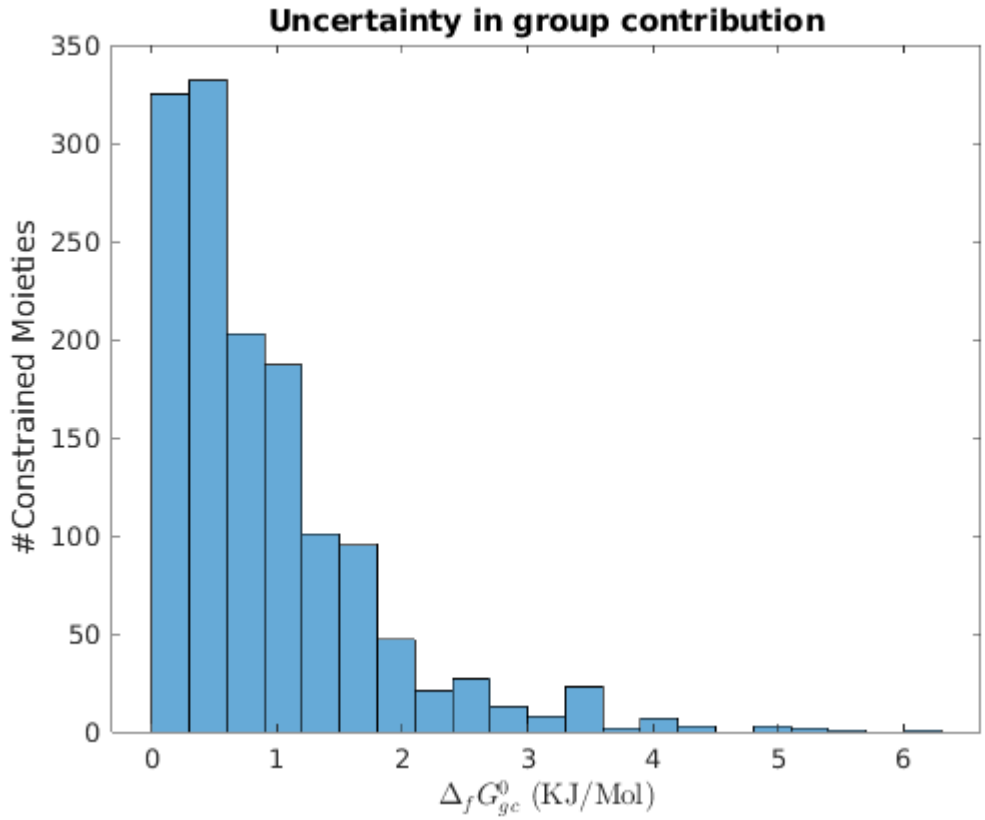
```
 fprintf('%u%s\n',nnz(solution.unconstrainedDfG0_gc),' of which DfG0_gc(j)
is unconstrained. i.e., group formation energies not constrained by group
contribution')
```

```
3788 of which DfG0_gc(j) is unconstrained. i.e., group formation energies not constrained by group contrib
```

```
 fprintf('%g%s\n',nnz(solution.unconstrainedDfG0_gc)/nGroups,' fraction of
unconstrained DfG0_gc')
```

```
0.730006 fraction of unconstrained DfG0_gc
```

```
figure
histogram(solution.DfG0_gc_Uncertainty(~solution.unconstrainedDfG0_gc))
title('Uncertainty in group contribution')
ylabel('#Constrained Moieties')
xlabel('$\Delta_{f} G^{0}_{gc}$ (KJ/Mol)','Interpreter','latex')
```




```
fprintf('%g%s\n',nnz(solution.DfG0_gc_Uncertainty==0 &
~solution.unconstrainedDfG0_gc),' number of zero uncertainty in constrained
DfG0_gc')
```

```
1 number of zero uncertainty in constrained DfG0_gc
```

```
fprintf('%g%s\n',nnz(solution.DfG0_gc_Uncertainty==0 &
solution.unconstrainedDfG0_gc),' number of zero uncertainty in unconstrained
DfG0_gc')
```

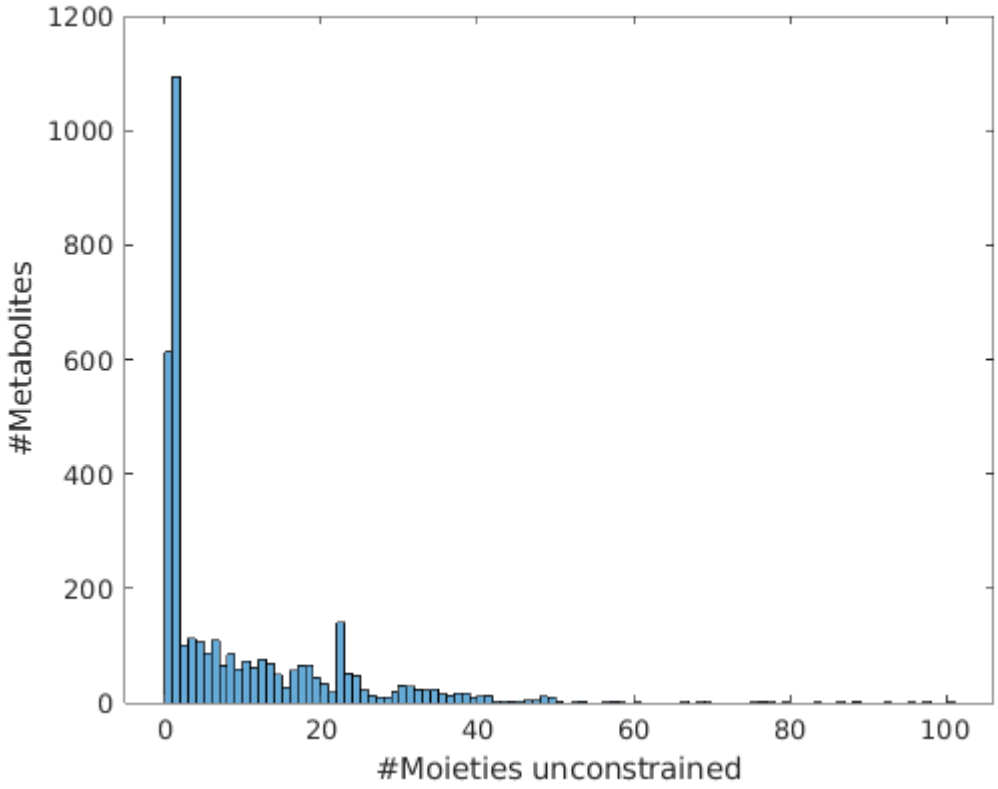
```
3788 number of zero uncertainty in unconstrained DfG0_gc
```

```
fprintf('%g%s\n',max(solution.DfG0_gc_Uncertainty),' maximum uncertainty for
any group.')
```

```
6.26551 maximum uncertainty for any group.
```

Analyse the number of metabolites with different numbers of unconstrained Moieties

```
nUnconstrainedGroupsPerMet = combinedModel.G*solution.unconstrainedDfG0_gc;
figure
histogram(nUnconstrainedGroupsPerMet,'BinWidth',1)
xlabel('#Moieties unconstrained')
ylabel('#Metabolites')
```



Conclusion: most metabolites only have one or two unconstrained Moieties.

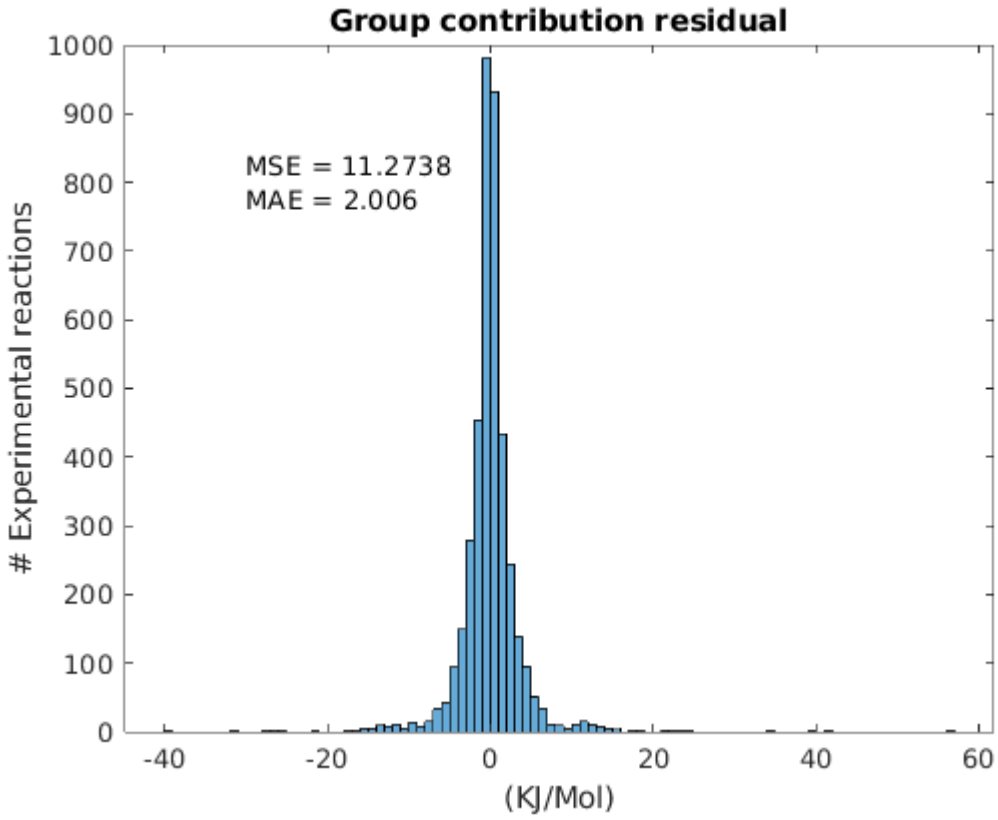
```
fprintf('%u%s\n',nnz(nUnconstrainedGroupsPerMet==0),' metabolites have no
unconstrained moieties')
```

```
613 metabolites have no unconstrained moieties
```

```
fprintf('%u%s\n',nnz(nUnconstrainedGroupsPerMet),' metabolites have at least
one unconstrained moiety')
```

```
3053 metabolites have at least one unconstrained moiety
```

```
figure;
histogram(solution.e_gc)
title('Group contribution residual')
xlabel('(KJ/Mol)');
ylabel('# Experimental reactions')
text(-30,800,{['MSE = ' num2str(solution.MSE_gc)],['MAE = '
num2str(solution.MAE_gc)]});
```

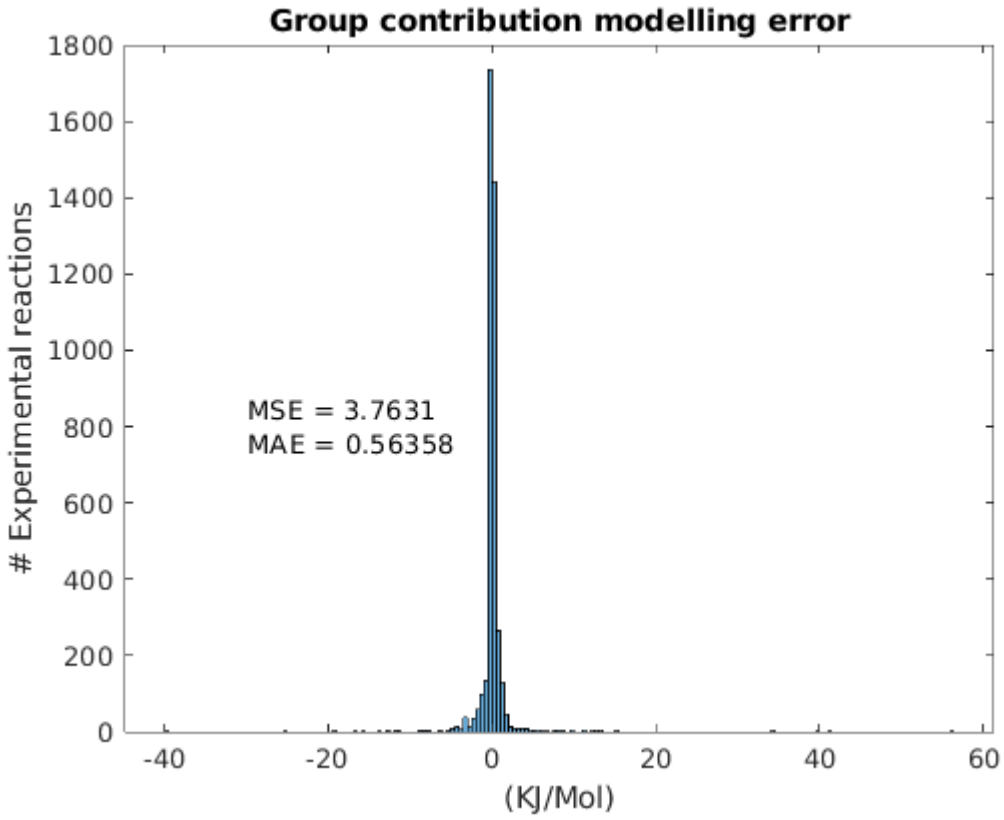



```
[gcErrorSorted,gcSI]=sort(solution.e_gc);
 N=10;
 for i=1:N
     rxnFormula =
printRxnFormula(combinedModel,'rxnAbbrList',combinedModel.rxns(gcSI(i)),'prin
tFlag',0);

fprintf('%g\t%s\t%s\n',solution.e_gc(rcSI(i)),combinedModel.rxns{gcSI(i)},rxn
Formula{1});
 end
```

```
-31.6414    FORM_C06670     -> C06670
-26.9545    TECRDB_1919    C00002 + C01281  -> C00013 + C01299
-21.9955    FORM_C00469     -> C00469
-25.3984    TECRDB_1929    C00002 + C01281  -> C00013 + C01299
-15.9678    FORM_C00093     -> C00093
-15.2367    TECRDB_1920    C00002 + C01281  -> C00013 + C01299
-15.6522    FORM_C00084     -> C00084
-13.3079    FORM_C80002     -> C80002
-15.3716    FORM_C01127     -> C01127
-12.4739    TECRDB_2655    C00003 + C00197  -> C00004 + C03232
```

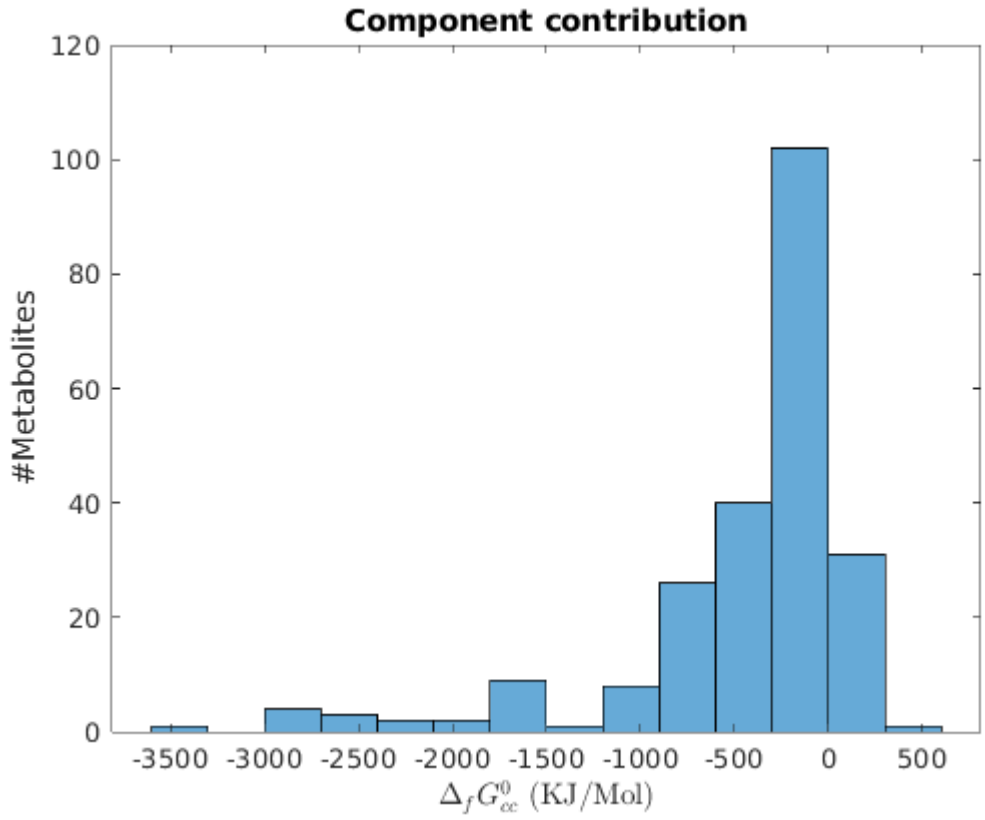
```
 figure;
 histogram(solution.e_m)
 title('Group contribution modelling error')
 xlabel('(KJ/Mol)');
 ylabel('# Experimental reactions')
```

```
 text(-30,800,{['MSE = ' num2str(solution.MSE_m)],['MAE = '
num2str(solution.MAE_m)]});
```



Analyse component contribution

```
figure
histogram(solution.DfG0_cc(~solution.unconstrainedDfG0_cc))
title('Component contribution')
ylabel('#Metabolites')
xlabel('$\Delta_{f} G^{0}_{cc}$ (KJ/Mol)','Interpreter','latex')
```




```
fprintf('%u%s\n',length(solution.DfG0_cc),' estimated reactant formation
energies.')
```

```
3666 estimated reactant formation energies.
```

```
fprintf('%u%s\n',nnz(solution.unconstrainedDfG0_cc),' of which DfG0_cc are
partially unconstrained by component contribution')
```

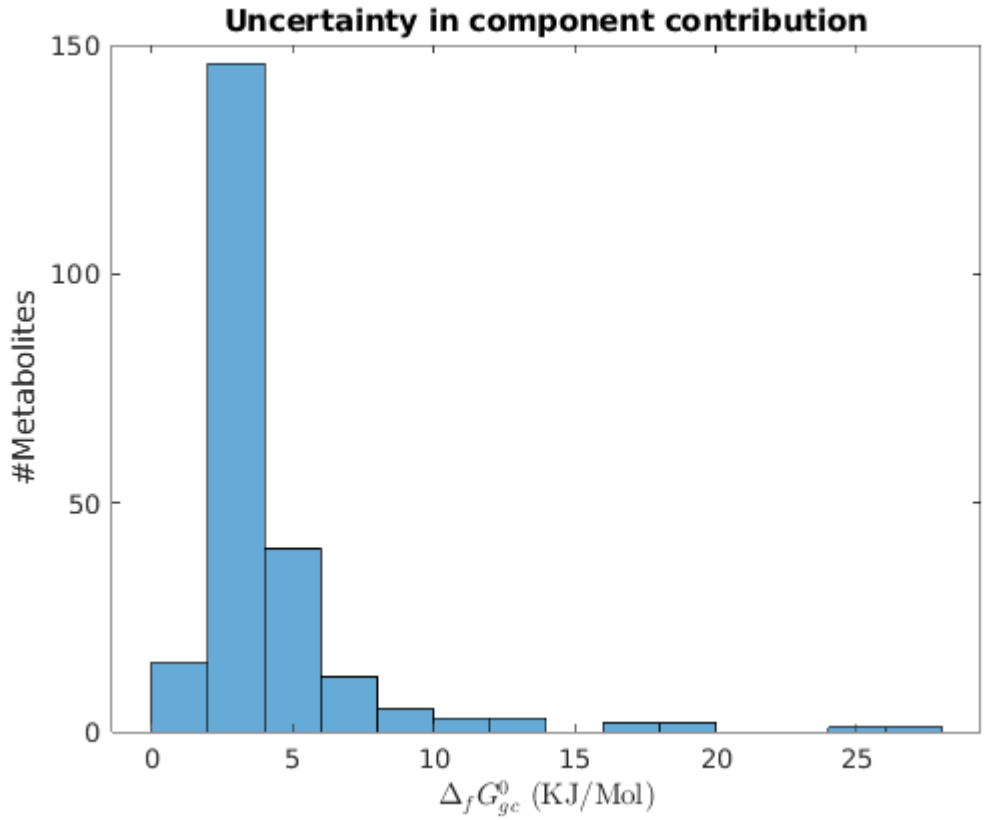
```
3436 of which DfG0_cc are partially unconstrained by component contribution
```

```
fprintf('%g%s\n',nnz(solution.unconstrainedDfG0_cc)/
length(solution.unconstrainedDfG0_cc),' = fraction
of partially unconstrained DfG0_cc')
```

```
0.937261 = fraction of partially unconstrained DfG0_cc
```

Uncertainty in component contribution

```
figure
histogram(solution.DfG0_cc_Uncertainty(~solution.unconstrainedDfG0_cc))
title('Uncertainty in component contribution')
ylabel('#Metabolites')
xlabel('$\Delta_{f} G^{0}_{gc}$ (KJ/Mol)','Interpreter','latex')
```



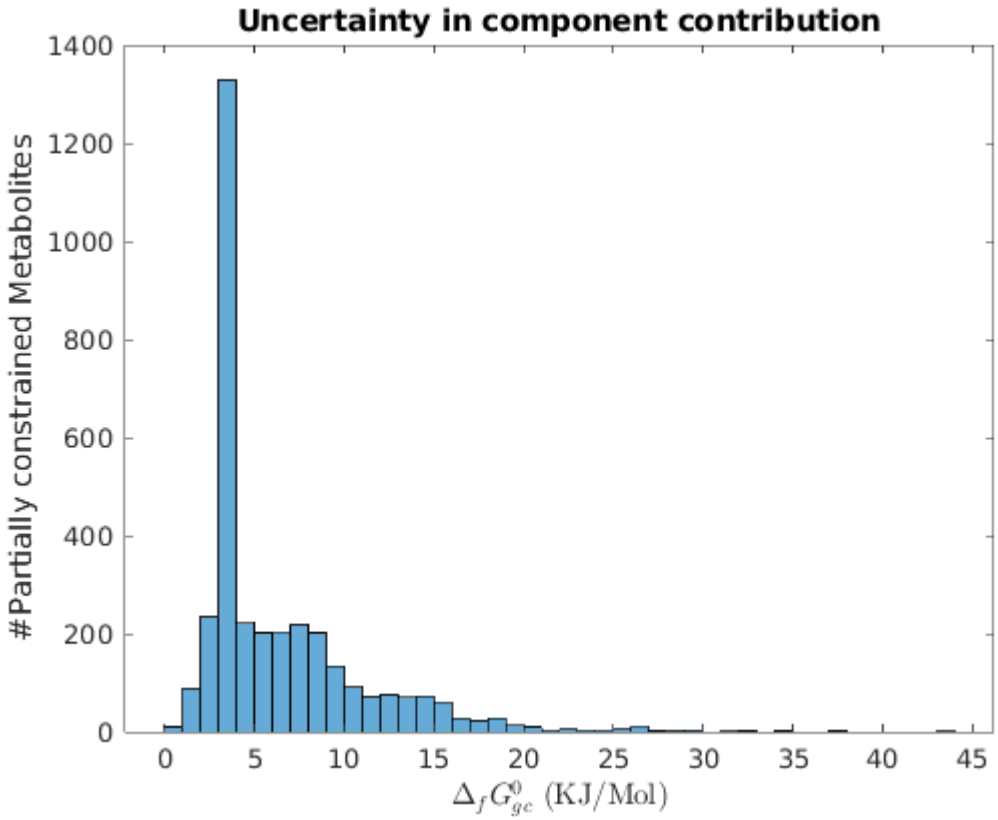

```
fprintf('%g%s\n',nnz(solution.DfG0_cc_Uncertainty==0 &
~solution.unconstrainedDfG0_cc),' number of zero uncertainty in constrained
DfG0_cc')
```

```
1 number of zero uncertainty in constrained DfG0_cc
```

```
fprintf('%g%s\n',nnz(solution.DfG0_cc_Uncertainty==0 &
solution.unconstrainedDfG0_cc),' number of zero uncertainty in unconstrained
DfG0_cc')
```

```
0 number of zero uncertainty in unconstrained DfG0_cc
```

```
figure
histogram(solution.DfG0_cc_Uncertainty(solution.unconstrainedDfG0_cc))
title('Uncertainty in component contribution')
ylabel('#Partially constrained Metabolites')
xlabel('$\Delta_{f} G^{0}_{gc}$ (KJ/Mol)','Interpreter','latex')
```

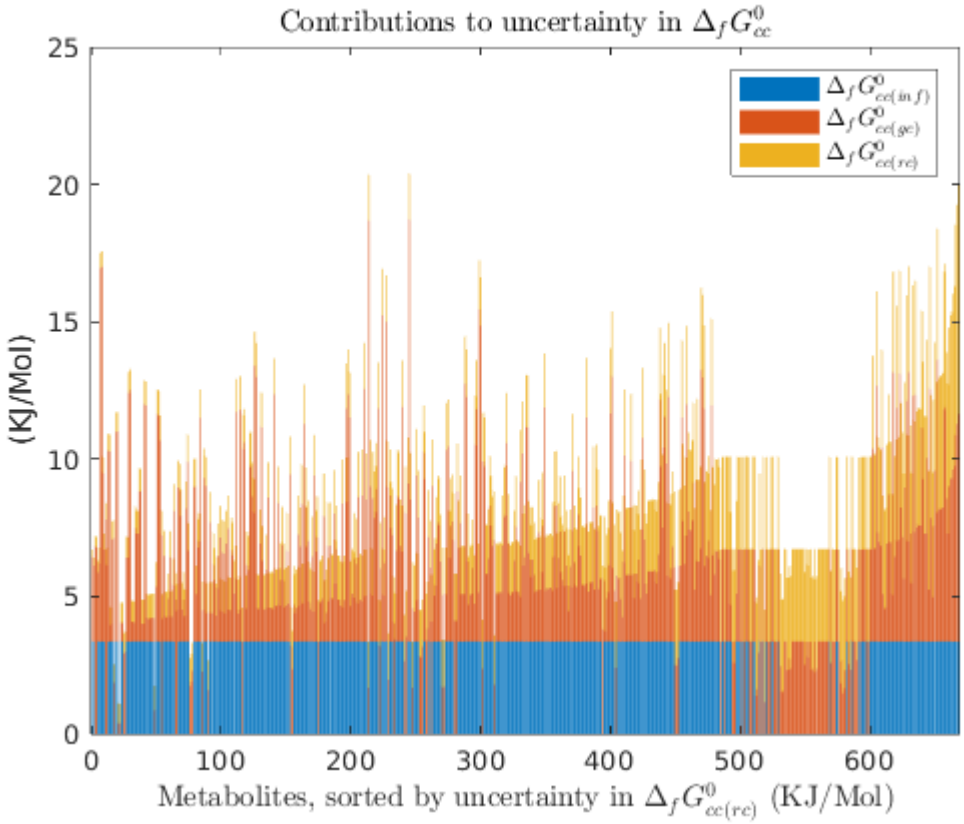


Breakdown of contributions to uncertainty in component contribution. Bar graph, with one bar for each reaction (row of the matrix). The height of each bar is the sum of the uncertainties in the reaction (row).

```
figure
Y =
[solution.DfG0_cc_inf_Uncertainty,solution.DfG0_cc_gc_Uncertainty,solution.Df
G0_rc_Uncertainty];
```

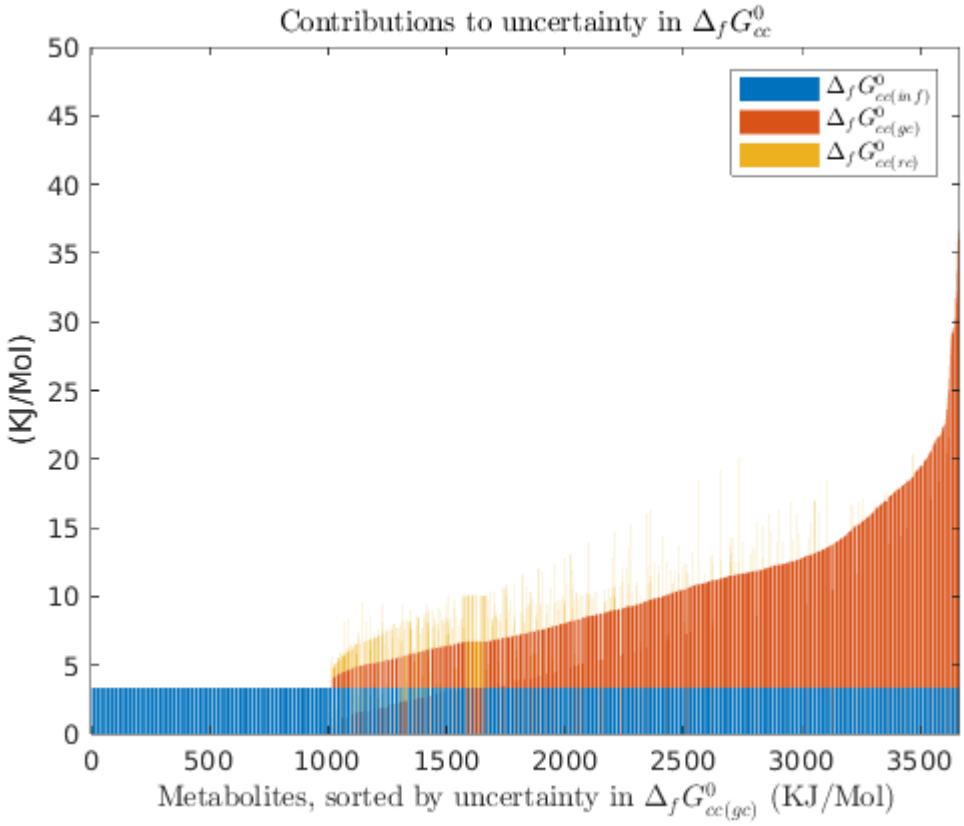
Sort by uncertainty in reactant contribution (for each metabolite)

```
[~,xi]=sort(solution.DfG0_rc_Uncertainty);
bar(Y(xi(solution.DfG0_rc_Uncertainty(xi)~=0),:),'stacked')
title('Contributions to uncertainty in $\Delta_{f} G^{0}_{cc}
$','Interpreter','latex')
xlabel('Metabolites, sorted by uncertainty in $\Delta_{f} G^{0}_{cc(rc)}$
(KJ/Mol)','Interpreter','latex')
ylabel('(KJ/Mol)')
legend({'$\Delta_{f} G^{0}_{cc(inf)}$','$\Delta_{f} G^{0}_{cc(gc)}$','$
\Delta_{f} G^{0}_{cc(rc)}$'},'Interpreter','latex')
```



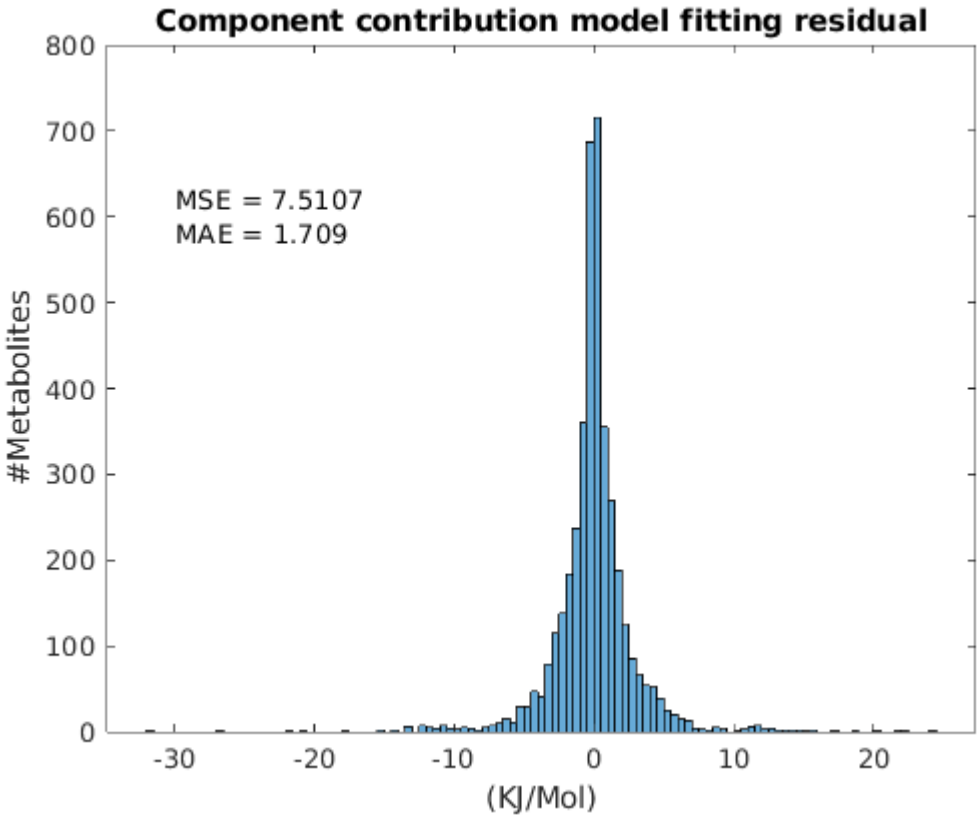
Sort by uncertainty in group contribution (for each metabolite)

```
[~,xi]=sort(solution.DfG0_cc_gc_Uncertainty);
bar(Y(xi,:),'stacked')
title('Contributions to uncertainty in $\Delta_{f} G^{0}_{cc}
$','Interpreter','latex')
xlabel('Metabolites, sorted by uncertainty in $\Delta_{f} G^{0}_{cc(gc)}$
(KJ/Mol)','Interpreter','latex')
ylabel('(KJ/Mol)')
legend({'$\Delta_{f} G^{0}_{cc(inf)}$','$\Delta_{f} G^{0}_{cc(gc)}$','$
\Delta_{f} G^{0}_{cc(rc)}$'},'Interpreter','latex')
```



Component contribution model fitting residual

```
figure;
histogram(solution.e_cc)
text(-30,600,{['MSE = ' num2str(solution.MSE_cc)],['MAE = '
num2str(solution.MAE_cc)]});
title('Component contribution model fitting residual')
xlabel('(KJ/Mol)')
ylabel('#Metabolites')
```




```
 [ccErrorSorted,ccSI]=sort(solution.e_cc);
 N=10;
 for i=1:N
     rxnFormula =
printRxnFormula(combinedModel,'rxnAbbrList',combinedModel.rxns(ccSI(i)),'prin
tFlag',0);

fprintf('%g\t%s\t%s\n',solution.e_cc(ccSI(i)),combinedModel.rxns{ccSI(i)},rxn
Formula{1});
 end
```

```
-31.6414    TECRDB_1919    C00002 + C01281  -> C00013 + C01299
-26.9545    TECRDB_1929    C00002 + C01281  -> C00013 + C01299
-21.9955    TECRDB_1920    C00002 + C01281  -> C00013 + C01299
-20.5893    FORM_C00093     -> C00093
-17.7694    FORM_C01127     -> C01127
-15.2367    TECRDB_539    C00002 + C00300  -> C00008 + C02305
-15.1381    TECRDB_2655    C00003 + C00197  -> C00004 + C03232
-14.375    TECRDB_3728    C00003 + C00469  -> C00004 + C00084
-14.2817    TECRDB_374    C00006 + C00342  -> C00005 + C00343
-13.4218    TECRDB_237    C00002 + C00062  -> C00008 + C05945
```

Comparison of weighting of reactant and group contribution for test metabolites

```
clear X
```

```
X(combinedModel.test2CombinedModelMap,:) = model.S;
XR = solution.PR_St*X;
XN = solution.PN_St*X;
XNR = (solution.PN_St - solution.PN_StGGt)*X;
XNN = solution.PN_StGGt*X;
```

Check that the decomposition into different components is complete

```
norm(X - (XR + XNR + XNN),'inf')
```

```
ans = 1.5682e-15
```

Stoichiometric degree

```
dX = diag(X*X');
fprintf('%u%s\n',nnz(dX),' metabolites with non-zero test stoichiometric
degree')
```

```
3128 metabolites with non-zero test stoichiometric degree
```

```
fprintf('%u%s\n',nnz(dX==0),' metabolites with zero test stoichiometric
degree')
```

```
538 metabolites with zero test stoichiometric degree
```

```
dXR = diag(XR*XR');
fprintf('%u%s\n',nnz(dX),' metabolites with non-zero test stoichiometric
degree, in the range of S''')
```

```
3128 metabolites with non-zero test stoichiometric degree, in the range of S'
```

```
fprintf('%u%s\n',nnz(dX==0),' metabolites with zero test stoichiometric
degree, in the range of S''')
```

```
538 metabolites with zero test stoichiometric degree, in the range of S'
```

```
dXN = diag(XN*XN');
fprintf('%u%s\n',nnz(dXN),' metabolites with non-zero test stoichiometric
degree, in the nullspace of S''')
```

```
3511 metabolites with non-zero test stoichiometric degree, in the nullspace of S'
```

```
fprintf('%u%s\n',nnz(dXN==0),' metabolites with zero test stoichiometric
degree, in the nullspace of S''')
```

```
155 metabolites with zero test stoichiometric degree, in the nullspace of S'
```

```
dXNR = diag(XNR*XNR');
fprintf('%u%s\n',nnz(dXNR),' metabolites with non-zero test stoichiometric
degree, in the nullspace of S'' and G''x in the range of G''S')
```

```
3470 metabolites with non-zero test stoichiometric degree, in the nullspace of S' and G'x in the range of
```

```
fprintf('%u%s\n',nnz(dXNR==0),' metabolites with zero test stoichiometric
degree, in the nullspace of S'' and G''x in the range of G''S')
```

```
196 metabolites with zero test stoichiometric degree, in the nullspace of S' and G'x in the range of G'S
```

```
dXNN = diag(XNN*XNN');
fprintf('%u%s\n',nnz(dXNN),' metabolites with non-zero test stoichiometric
degree, in the nullspace of S'' and in the nullspace of S''GG'' ')
```

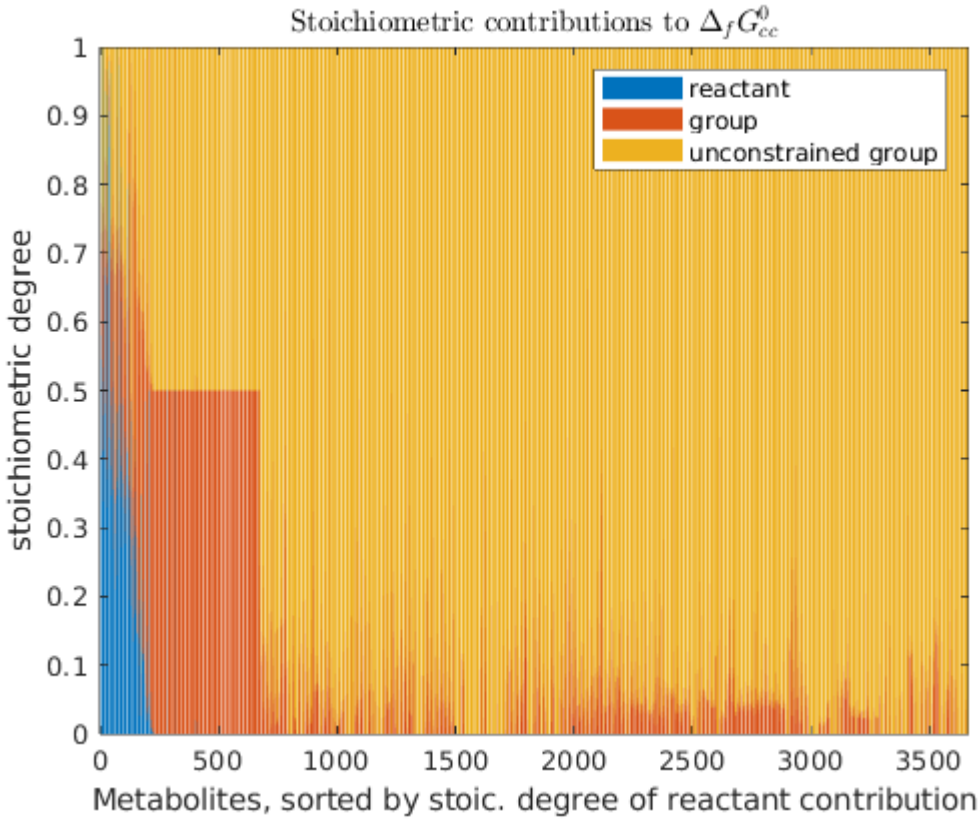
```
3656 metabolites with non-zero test stoichiometric degree, in the nullspace of S' and in the nullspace of
```

```
fprintf('%u%s\n',nnz(dXNN==0),' metabolites with zero test stoichiometric
degree, in the nullspace of S'' and in the nullspace of S''GG'' ')
```

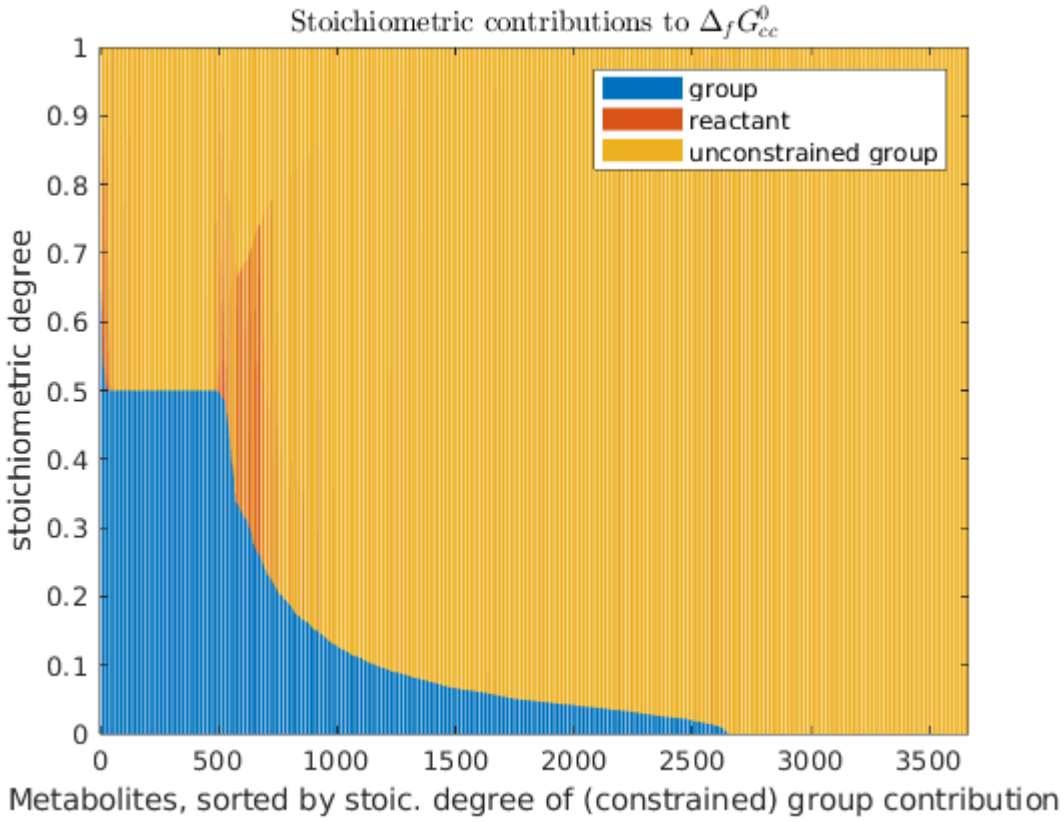
```
10 metabolites with zero test stoichiometric degree, in the nullspace of S' and in the nullspace of S'GG'
```

Sort by stoichiometric degree of reactant contribution (for each metabolite)

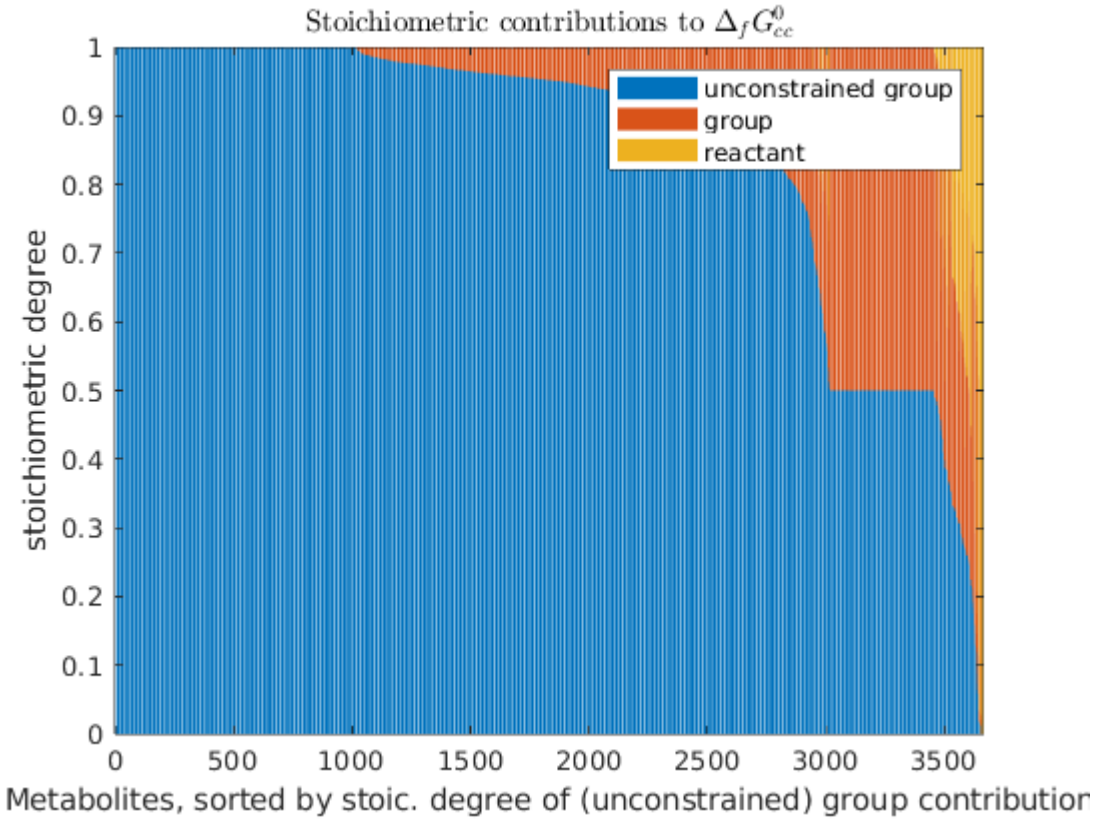
```
dXtotal = dXR + dXNR + dXNN;
Y = [dXR./dXtotal,dXNR./dXtotal,dXNN./dXtotal];
[dXRsorted,xi]=sort(dXR,'descend');
figure
bar(Y(xi,:),'stacked')
ylim([0 1])
title('Stoichiometric contributions to $\Delta_{f} G^{0}_{cc}
$','Interpreter','latex')
xlabel('Metabolites, sorted by stoic. degree of reactant contribution')
ylabel('stoichiometric degree')
legend({'reactant','group','unconstrained group'})
```




```
Y = [dXNR./dXtotal,dXR./dXtotal,dXNN./dXtotal];
[~,xi]=sort(dXNR./dXtotal,'descend');
figure
bar(Y(xi,:),'stacked')
ylim([0 1])
title('Stoichiometric contributions to $\Delta_{f} G^{0}_{cc}
$','Interpreter','latex')
xlabel('Metabolites, sorted by stoic. degree of (constrained) group
contribution')
ylabel('stoichiometric degree')
legend({'group','reactant','unconstrained group'})
```

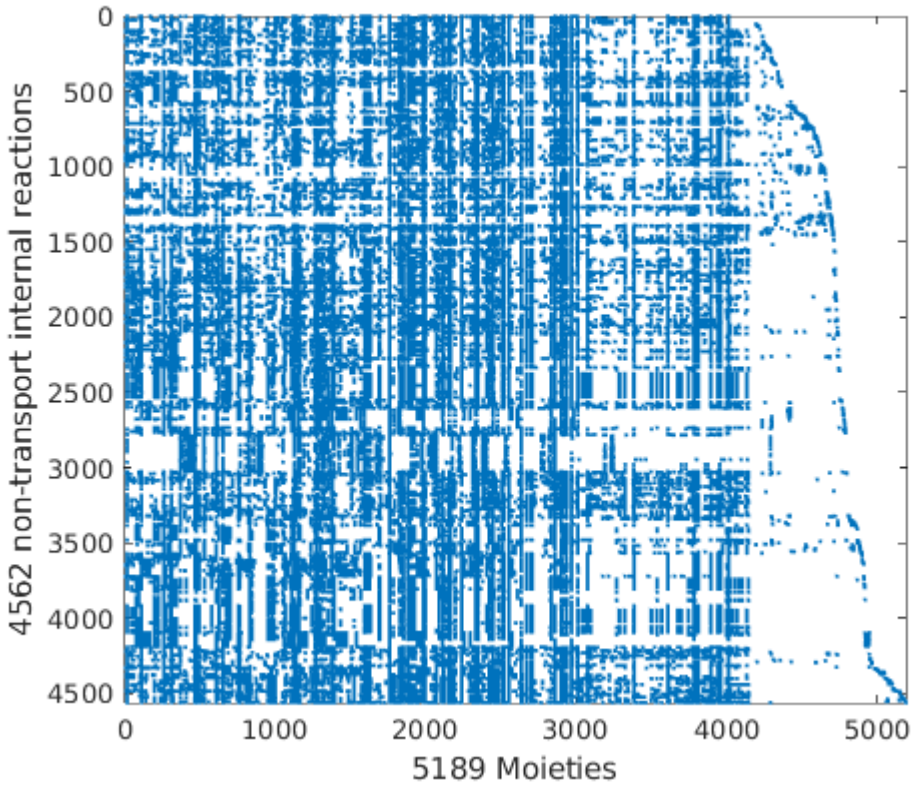



```
Y = [dXNN./dXtotal,dXNR./dXtotal,dXR./dXtotal];
[~,xi]=sort(dXNN./dXtotal,'descend');
figure
bar(Y(xi,:),'stacked')
ylim([0 1])
title('Stoichiometric contributions to $\Delta_{f} G^{0}_{cc}
$','Interpreter','latex')
xlabel('Metabolites, sorted by stoic. degree of (unconstrained) group
contribution')
ylabel('stoichiometric degree')
legend({'unconstrained group','group','reactant'})
```

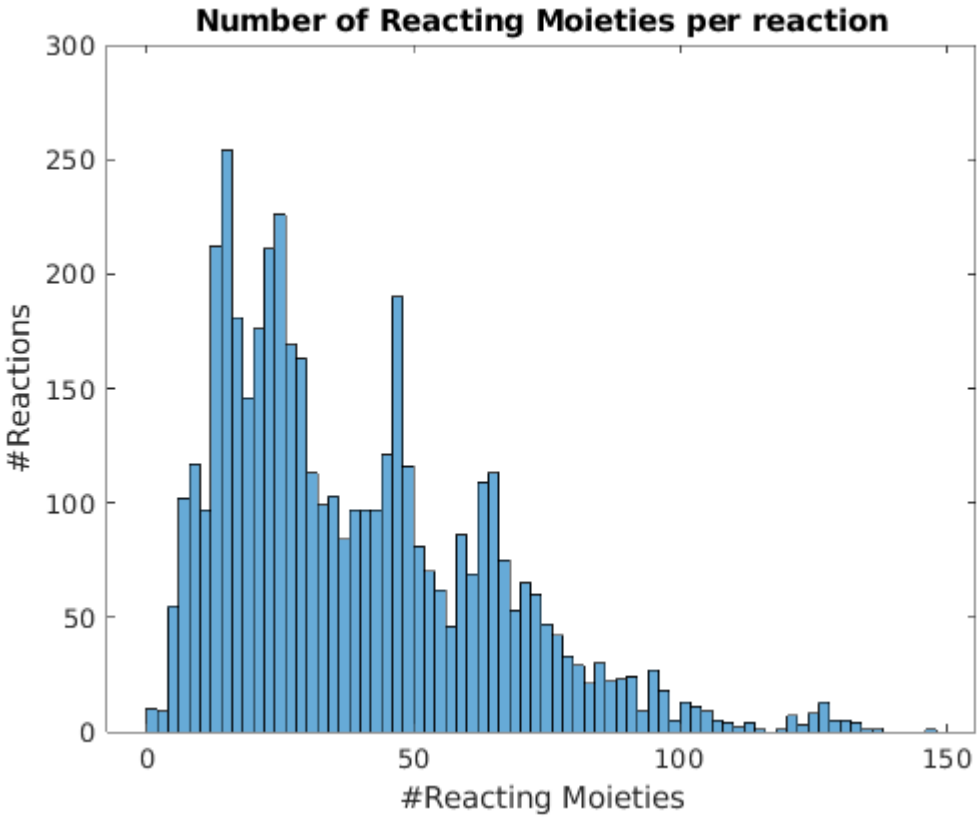


Analyse reacting moieties in the test model

```
transportRxnBool = transportReactionBool(model);
model.G=combinedModel.G(combinedModel.test2CombinedModelMap,:);
StG=model.S'*model.G;
bool = model.SIntRxnBool & ~transportRxnBool;
figure
spy(StG(bool,:))
xlabel([int2str(size(StG(bool,:),2)) ' Moieties'])
ylabel([int2str(size(StG(bool,:),1)) ' non-transport internal reactions'])
```




```
nReactingMoieties=full(sum(StG~=0,2));
histogram(nReactingMoieties(bool),'BinWidth',2)
title('Number of Reacting Moieties per reaction')
xlabel('#Reacting Moieties')
ylabel('#Reactions')
```



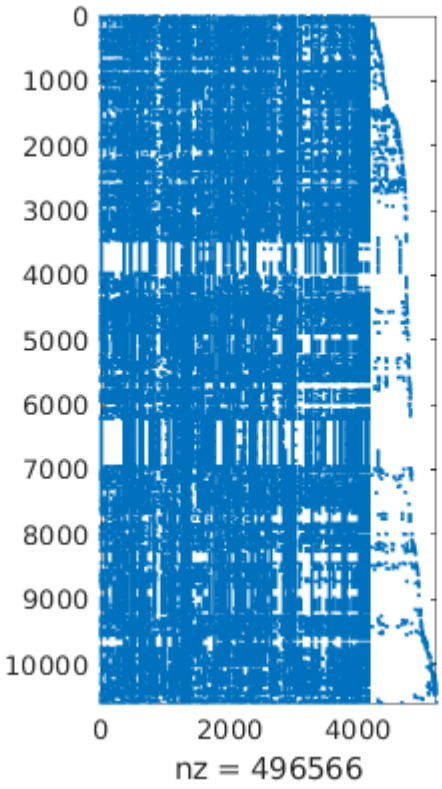

```
fprintf('%u%s\n',nnz(nReactingMoieties==0 & bool),' internal non-transport
reactions without reacting moieties.')
```

```
10 internal non-transport reactions without reacting moieties.
```

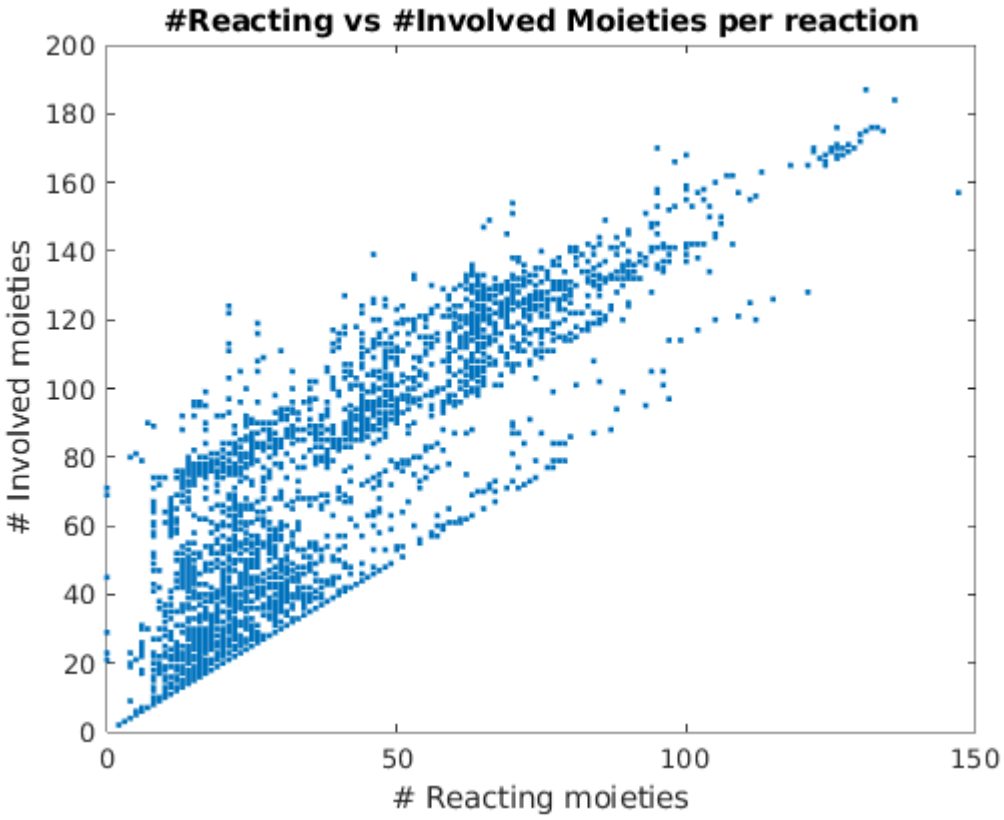
```
printRxnFormula(model,model.rxns(bool & nReactingMoieties==0));
```

```
CYSGLTH    Lcystin[c] + 2 gthrd[c]     <=>    2 cys_L[c] + gthox[c]
NDPK8m    atp[m] + dadp[m]     ->    adp[m] + datp[m]
NDPK8n    atp[n] + dadp[n]     <=>    datp[n] + adp[n]
RAI2    retinal[c]     <=>    retinal_cis_9[c]
RETI2    retinol[c]     <=>    retinol_9_cis[c]
TMDPPK    atp[c] + thmpp[c]     ->    adp[c] + thmtp[c]
RE2651R    retinal[r]     <=>    retinal_cis_9[r]
RE3002X    CE5114[x]     <=>    CE5116[x]
NDPK8    atp[c] + dadp[c]     <=>    adp[c] + datp[c]
HMR_3447    CE5116[m]     ->    CE5114[m]
```

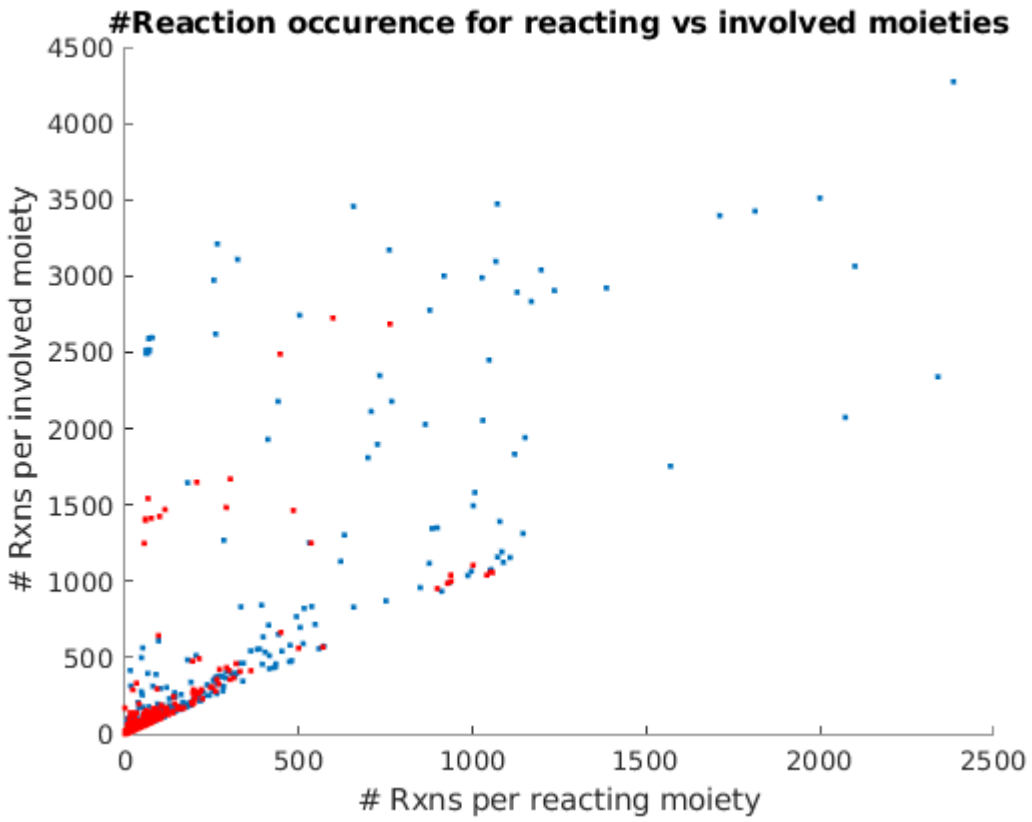
```
AtG=(model.S~=0)'*model.G;
spy(AtG)
```




```
nInvolvedMoieties=full(sum(AtG~=0,2));
bool = model.SIntRxnBool & ~transportRxnBool;
plot(nReactingMoieties(bool),nInvolvedMoieties(bool),'.')
xlabel('# Reacting moieties')
ylabel('# Involved moieties')
title('#Reacting vs #Involved Moieties per reaction')
```




```
nRxnsMoietiesInvolved=full(sum(AtG(bool,:)~=0,1)');
nRxnsMoietiesReacting=full(sum(StG(bool,:)~=0,1)');
figure
hold on
plot(nRxnsMoietiesReacting,nRxnsMoietiesInvolved,'.')
plot(nRxnsMoietiesReacting(solution.unconstrainedDfG0_gc),nRxnsMoietiesInvolv
ed(solution.unconstrainedDfG0_gc),'.r')
hold off
xlabel('# Rxns per reacting moiety')
ylabel('# Rxns per involved moiety')
title('#Reaction occurence for reacting vs involved moieties')
```

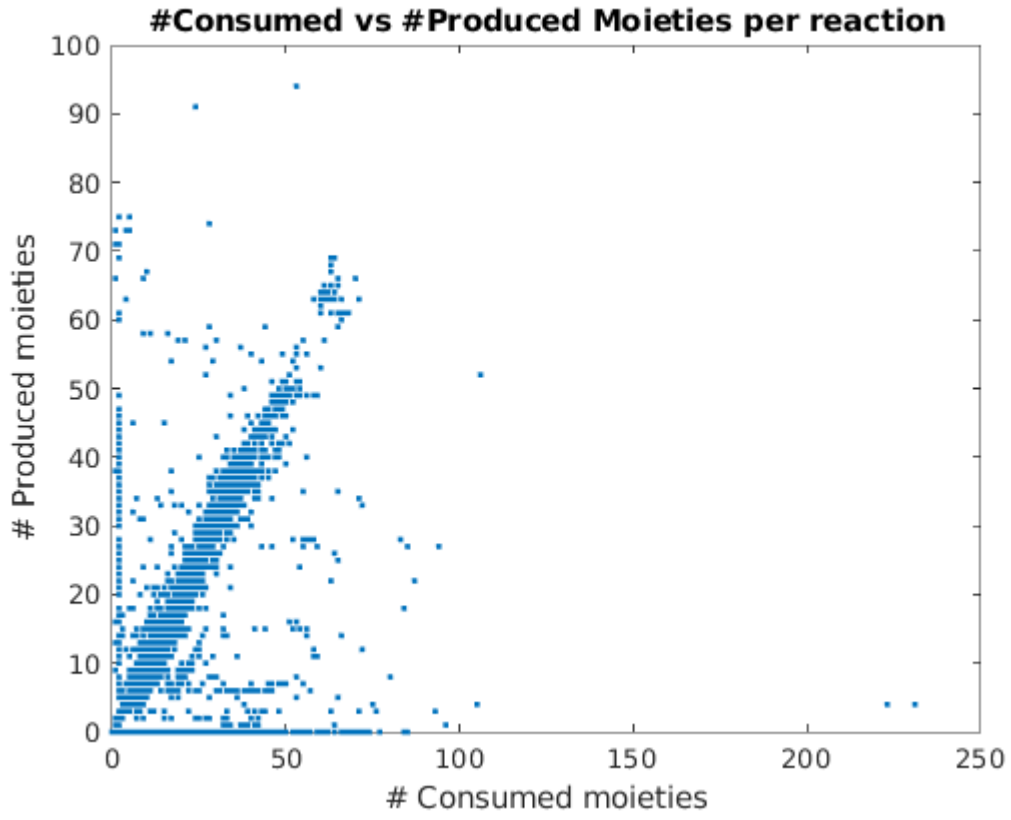


Moieties reacting in a lot of reactions but unconstrained by group contribution

```
T = table(combinedModel.groups(nRxnsMoietiesReacting>1000 &
solution.unconstrainedDfG0_gc),nRxnsMoietiesReacting(nRxnsMoietiesReacting>10
00 & solution.unconstrainedDfG0_gc),'VariableNames',
{'Reacting_Moiety','#Reactions'});
disp(T)
```

Reacting_Moiety	#Reactions
{'C/C(=N)[O-]' }	1057
{'C=C(C)/C(=N)[O-]' }	1041
{'CCC(=CN)/C(=N)[O-]' }	1041
{'C[C@@H](n)O' }	1001
{'c/C(=N)[O-]' }	1054
{'cc(c)/C(=N)[O-]' }	1052
{'ccc(c[n+])/C(=N)[O-]'}	1052

```
nConsumedMoieties=sum(StG<0,2);
nProducedMoieties=sum(StG>0,2);
plot(nConsumedMoieties,nProducedMoieties,'.')
xlabel('# Consumed moieties')
ylabel('# Produced moieties')
title('#Consumed vs #Produced Moieties per reaction')
```



Reaction Component contribution taking into account reacting moieties only

```
nnz(solution.unconstrainedDfG0_cc)
```

```
ans = 3436
```

```
bool = model.SIntRxnBool & ~transportRxnBool;
fprintf('%u%s\n',nnz(bool),' internal non-transport reactions.')
```

```
4562 internal non-transport reactions.
```

```
fprintf('%u%s\n',nnz(model.unconstrainedDrG0_cc & bool),' of which have
unconstrained DrG0.')
```

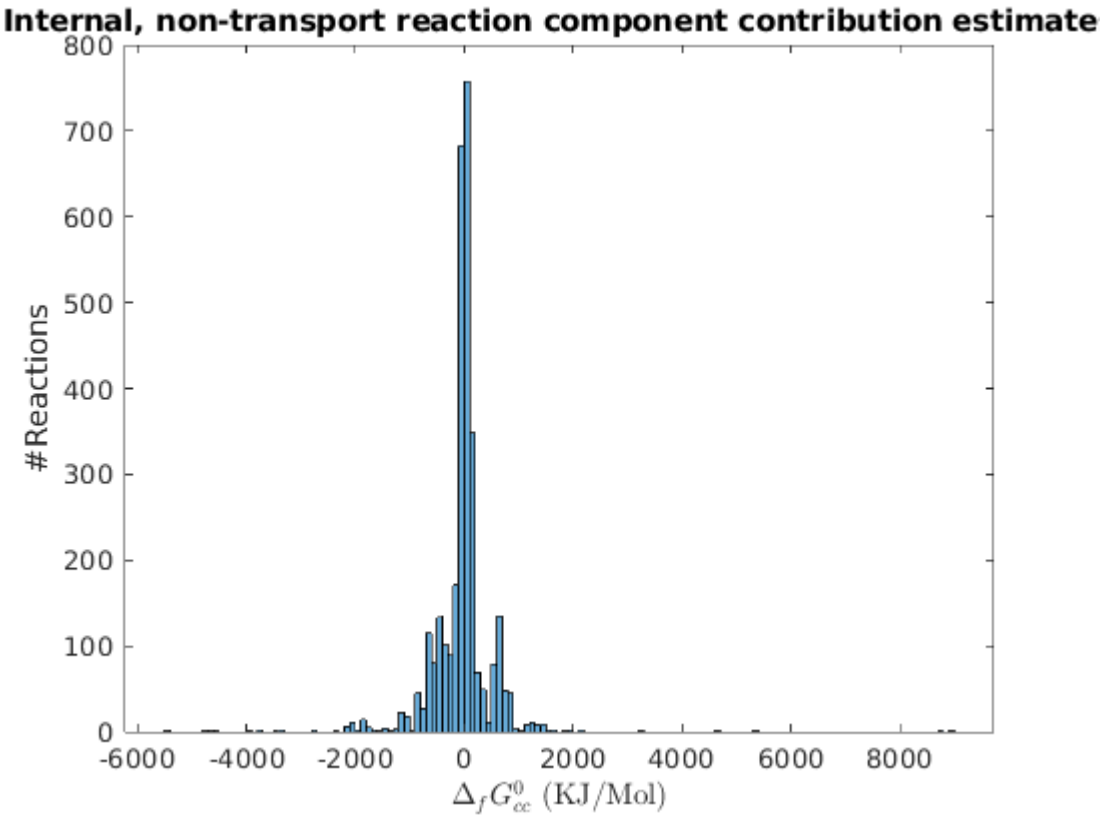
```
1419 of which have unconstrained DrG0.
```

```
ind=find(model.unconstrainedDrG0_cc & bool);
```

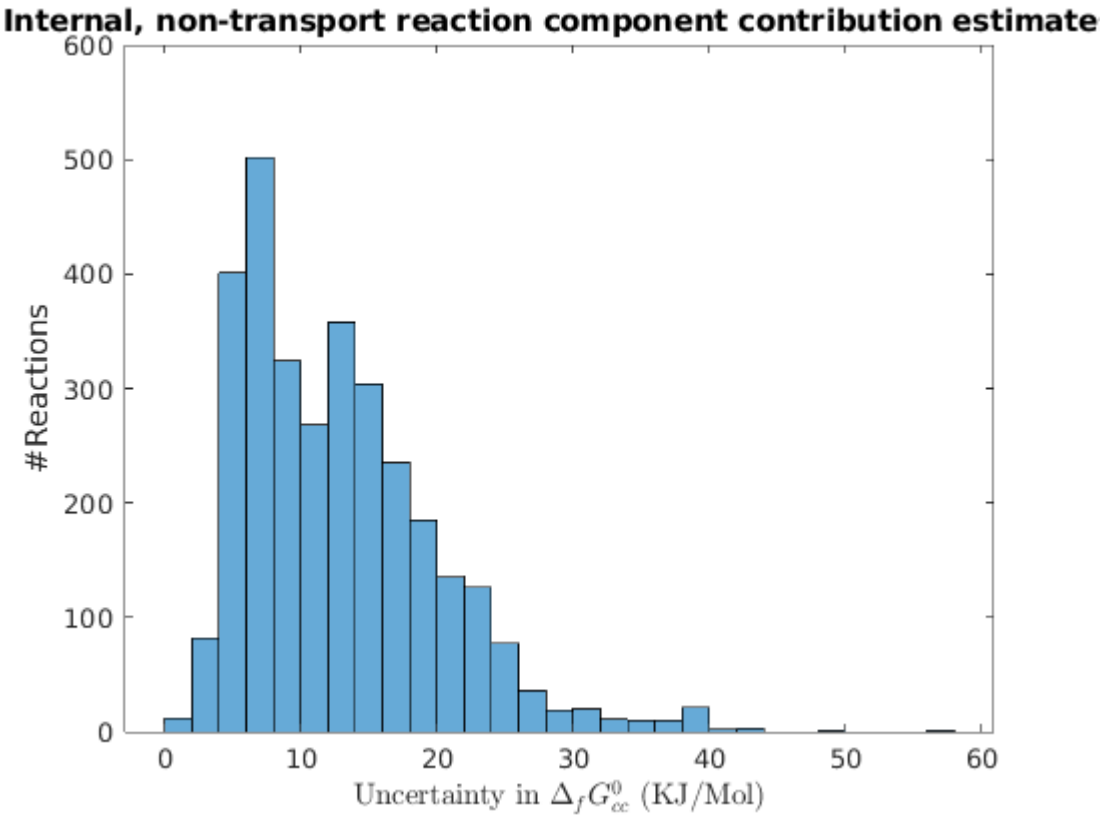
Estimated standard reaction Gibbs energy

```
figure;
bool = model.SIntRxnBool & ~transportRxnBool & ~model.unconstrainedDrG0_cc;
histogram(model.DrG0(bool))
title('Internal, non-transport reaction component contribution estimates')
```

```
  ylabel('#Reactions')
  xlabel('$\Delta_{f} G^{0}_{cc}$ (KJ/Mol)','Interpreter','latex')
```



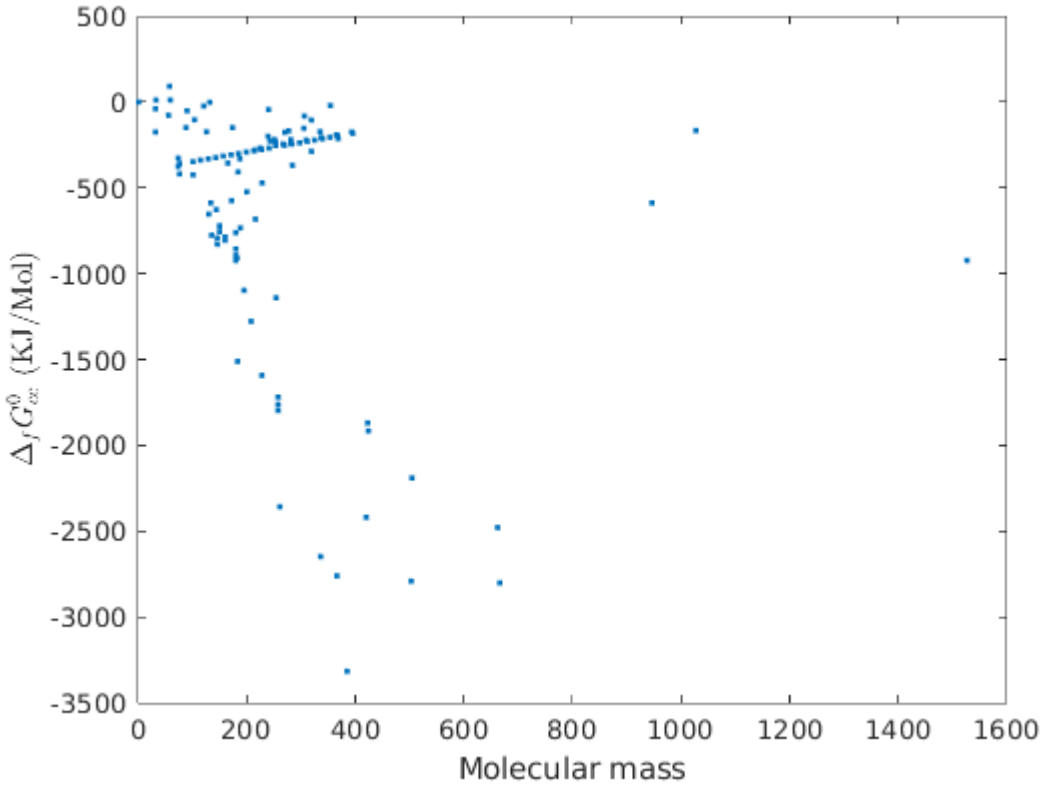

```
  figure;
  bool = model.SIntRxnBool & ~transportRxnBool & ~model.unconstrainedDrG0_cc;
  histogram(model.DrG0_Uncertainty(bool))
  title('Internal, non-transport reaction component contribution estimates')
  ylabel('#Reactions')
  xlabel('Uncertainty in $\Delta_{f} G^{0}_{cc}$ (KJ/
Mol)','Interpreter','latex')
```



Estimated standard metabolite Gibbs energy vs molecular mass

```
figure
[metMasses, knownMasses, unknownElements, Ematrix, elements] =
getMolecularMass(model.metFormulas);
```

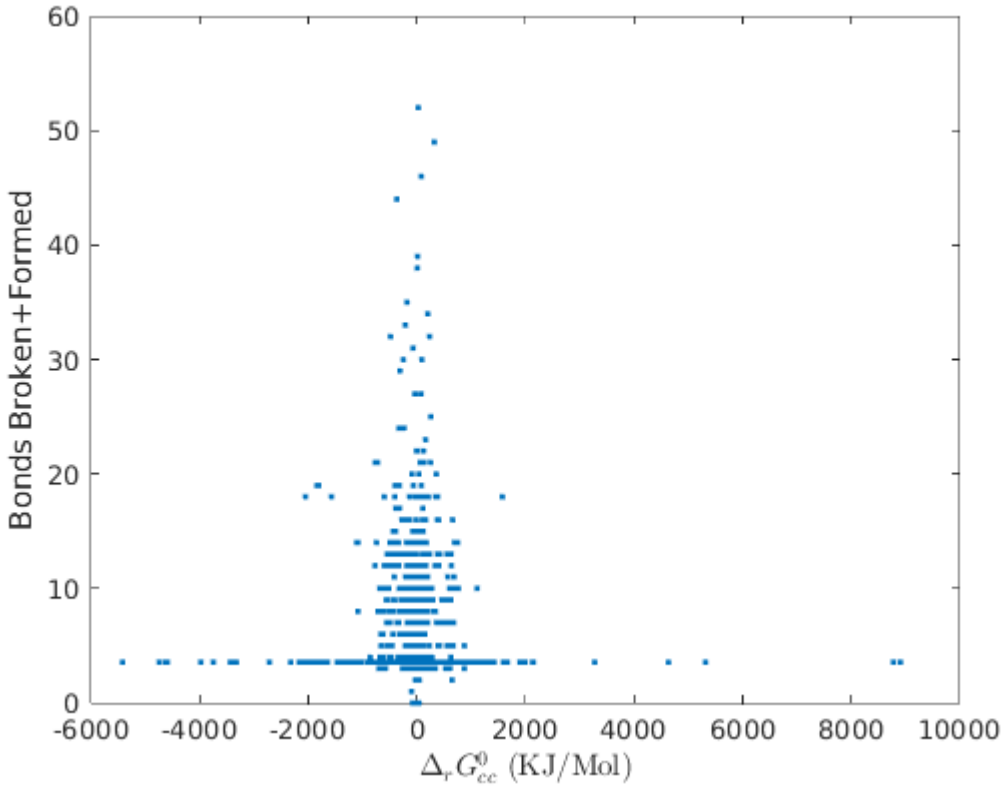
```
plot(metMasses(~model.unconstrainedDfG0_cc),model.DfG0(~model.unconstrainedDf
G0_cc),'.')
xlabel('Molecular mass')
ylabel('$\Delta_{f} G^{0}_{cc}$ (KJ/Mol)','Interpreter','latex')
```



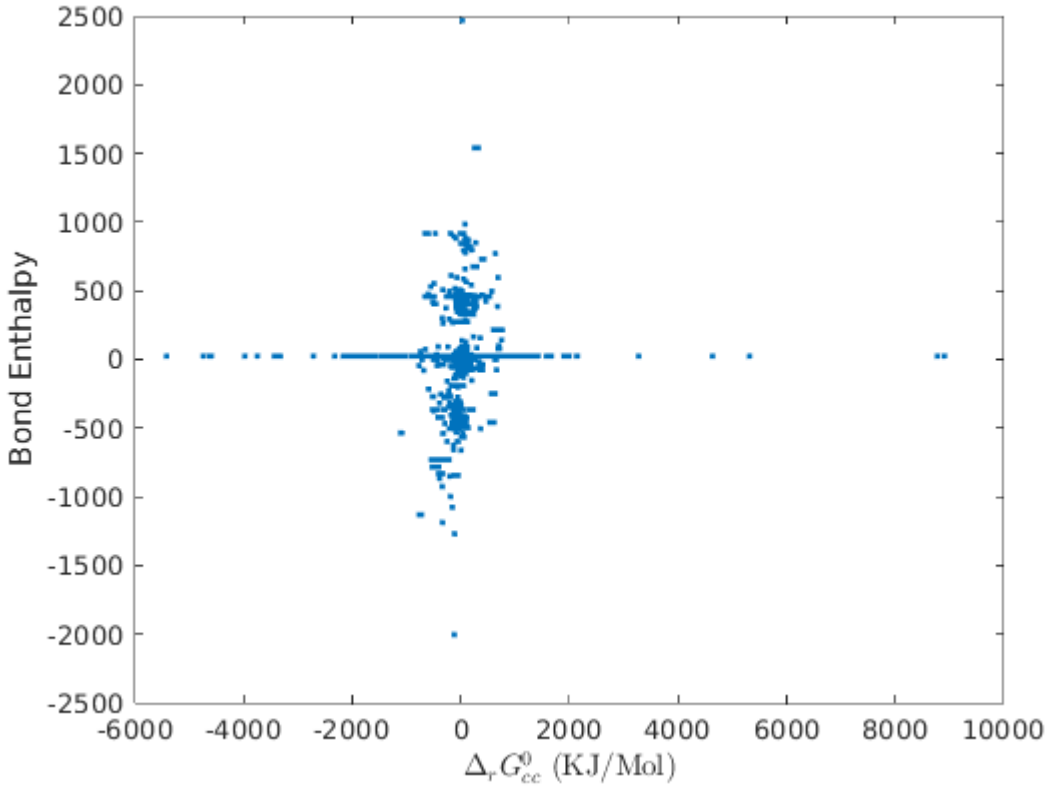
Estimated standard reaction Gibbs energy vs bonds broken and formed

```
inputFolder = ['~' filesep 'work' filesep 'sbgCloud' filesep
'programExperimental' filesep 'projects' filesep 'xomics' filesep 'data'
filesep 'Recon3D_301'];
BBFmodel = load([inputFolder filesep 'Recon3DModel_301_thermo_BBF.mat']);
%BBFmodel = load('~/work/sbgCloud/programExperimental/projects/xomics/data/
Recon3D_301/Recon3DModel_301_thermo_BBF');
BBFmodel=BBFmodel.model;
```

```
model.transportRxnBool = transportReactionBool(model);
bool = ~model.unconstrainedDrG0_cc & model.SIntRxnBool &
~model.transportRxnBool;
DfG0_cc = solution.DfG0_cc(combinedModel.test2CombinedModelMap);
DrG0_cc = model.S'*DfG0_cc;
figure
plot(DrG0_cc(bool),BBFmodel.bondsBF(bool),'.')
ylabel('Bonds Broken+Formed')
xlabel('$\Delta_{r} G^{0}_{cc}$ (KJ/Mol)','Interpreter','latex')
```




```
plot(DrG0_cc(bool),BBFmodel.bondsE(bool),'.')
ylabel('Bond Enthalpy')
xlabel('$\Delta_{r} G^{0}_{cc}$ (KJ/Mol)','Interpreter','latex')
```




```
% unconstrainedDfG0_cc = model.S
%
% %DfG0_cc = PR_St * DfG0_rc + PN_St * G * DfG0_gc;
% DfG0_cc = PR_St * DfG0_rc + PN_St * G * DfG0_gc;
% model.PR_St=solution.PR_St(combinedModel.test2CombinedModelMap,:);
% model.PN_St=solution.PN_St(combinedModel.test2CombinedModelMap,:);
% DrG0_rc = model.S'*model.PR_St*solution.DfG0_rc + model.S'*model.PN_St *
solution.G * solution.DfG0_gc;
%
% %identify the component contribution estimates that are unconstrained
% reactantContUnconstrainedDfG0_cc = (PR_St * unconstrainedDfG0_rc)~=0;
% groupContUnconstrainedDfG0_cc = (PN_St * G * unconstrainedDfG0_gc)~=0;
% unconstrainedDfG0_cc = (PR_St * unconstrainedDfG0_rc + PN_St * G *
unconstrainedDfG0_gc)~=0;
```

```
% DrG0_cc = model.S'*model.PR_St*solution.DfG0_rc + model.S'*model.PN_St *
solution.G * solution.DfG0_gc;
```