Extract a thermodynamically consistent subnetwork from a given model

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Reviewer(s):

1. Identify the largest subset of a model that admits a thermodynamically consistent flux
2. Specify a random subset of active/inactive reactions and present/absent metabolites
3. Compute the smallest thermodynamically consistent subnetwork containing a list of present metabolites and active reactions, and not containing a list of absent metabolites and inactive reactions

```
[solverOK,solverInstalled]=changeCobraSolver('ibm_cplex','all');
```

```
> changeCobraSolver: IBM ILOG CPLEX interface added to MATLAB path.
> ibm_cplex (version 1210) is compatible and fully tested with MATLAB R2021a on your operating system.
> changeCobraSolver: Solver for LP problems has been set to ibm_cplex.

> changeCobraSolver: IBM ILOG CPLEX interface added to MATLAB path.
> ibm_cplex (version 1210) is compatible and fully tested with MATLAB R2021a on your operating system.
> changeCobraSolver: Solver for MILP problems has been set to ibm_cplex.

> changeCobraSolver: IBM ILOG CPLEX interface added to MATLAB path.
> ibm_cplex (version 1210) is compatible and fully tested with MATLAB R2021a on your operating system.
> changeCobraSolver: Solver for QP problems has been set to ibm_cplex.

> changeCobraSolver: IBM ILOG CPLEX interface added to MATLAB path.
> ibm_cplex (version 1210) is compatible and fully tested with MATLAB R2021a on your operating system.
> changeCobraSolver: Solver for MIQP problems has been set to ibm_cplex.
> changeCobraSolver: Solver ibm_cplex not supported for problems of type EP. No solver set for this probl
> changeCobraSolver: Solver ibm_cplex not supported for problems of type NLP. No solver set for this prob
```

```
%[solverOK,solverInstalled]=changeCobraSolver('gurobi','all');
%[solverOK,solverInstalled]=changeCobraSolver('ibm_cplex','QP');
```

Load model

```
modelToLoad='circularToy';
modelToLoad='ecoli_core';
modelToLoad='modelRecon3MitoOpen';
modelToLoad='Recon3DModel';
%modelToLoad='iDopa';
```

Load a model

```
driver_thermoModelLoad
```

```
Model loaded: Recon3DModel
lower bounds greater than zero
Internal stochiometric nullspace computed in 4.5238 seconds.
```

Stoichiometric consistency

```
if ~isfield(model,'SConsistentRxnBool')  ||
~isfield(model,'SConsistentMetBool')
    massBalanceCheck=0;
    %massBalanceCheck=1;
    printLevel=2;
    [SConsistentMetBool, SConsistentRxnBool,
SInConsistentMetBool, SInConsistentRxnBool, unknownSConsistencyMetBool,
unknownSConsistencyRxnBool, model,stoichConsistModel]...
        = findStoichConsistentSubset(model, massBalanceCheck, printLevel);
else
    %Extract stoich consistent submodel
    if any(~model.SConsistentMetBool)
        rxnRemoveMethod='inclusive';%maintains stoichiometric consistency
        [stoichConsistModel, rxnRemoveList] = removeMetabolites(model,
model.mets(~model.SConsistentMetBool),rxnRemoveMethod);
        SConsistentRxnBool2=~ismember(model.rxns,rxnRemoveList);
        if ~all(model.SConsistentRxnBool==SConsistentRxnBool2)
            error('inconsistent reaction removal')
        end
        try
            stoichConsistModel = removeUnusedGenes(stoichConsistModel);
        catch ME
            disp(ME.message)
        end
    else
        stoichConsistModel = model;
    end
end

[nMet,nRxn]=size(stoichConsistModel.S)
```

```
nMet = 5835
nRxn = 10600
```

Flux consistency

```
fluxConsistentParam.method='fastcc';%can handle additional constraints
fluxConsistentParam.printLevel=1;
[~,~,~,~,stoichConsistModel]=
findFluxConsistentSubset(stoichConsistModel,fluxConsistentParam);
```

Extract flux consistent submodel

```
if any(~stoichConsistModel.fluxConsistentRxnBool)
    rxnRemoveList =
stoichConsistModel.rxns(~stoichConsistModel.fluxConsistentRxnBool);
```

```
    stoichFluxConsistModel = removeRxns(stoichConsistModel,
rxnRemoveList,'metRemoveMethod','exclusive','ctrsRemoveMethod','inclusive');
    try
        stoichFluxConsistModel = removeUnusedGenes(stoichFluxConsistModel);
        catch ME
        disp(ME.message)
    end
else
    stoichFluxConsistModel = stoichConsistModel;
end
[nMet,nRxn]=size(stoichFluxConsistModel.S)
```

```
nMet = 5835
nRxn = 10600
```

Thermodynamic consistency

```
%save('debug_prior_to_findThermoConsistentFluxSubset.mat')
%return
param.printLevel = 1;
param.relaxBounds=0;
param.acceptRepairedFlux=1;
[thermoFluxConsistentMetBool,thermoFluxConsistentRxnBool,stoichFluxConsistMod
el,stoichFluxThermoConsistModel] =
findThermoConsistentFluxSubset(stoichFluxConsistModel,param);
```

```
--- findThermoFluxConsistentSubset START ----
                printLevel: 1
               relaxBounds: 0
         acceptRepairedFlux: 1
                    epsilon: 1.0000e-06
               formulation: 'pqzw'
           iterationMethod: 'random'
                      nMax: 20
           warmStartMethod: 'random'
           thetaMultiplier: 1.5000
                     theta: 0.5000
           regularizeOuter: 0
    thermoConsistencyMethod: 'cycleFreeFlux'
                   bigNum: 10000
                    debug: 0

optCardThermo objective data:
         0.1 = beta, the global weight on one-norm of internal reaction rate.
          -5 = min(g0), the local weight on zero-norm of internal reaction rate.
          -0 = max(g0), the local weight on zero-norm of internal reaction rate.
           0 = min(h0), the local weight on zero-norm of metabolite production rate.
           0 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

0 min cardinality variables:
         NaN mean(c(p))             NaN min(c(p))             NaN max(c(p))
           1 lambda0             NaN min(k)             NaN max(k)
           1 lambda1             NaN min(o(p))             NaN max(o(p))

5303 max cardinality variables:
```

```
          -0 mean(c(q))              -0 min(c(q))              -0 max(c(q))
           1 delta0                5 min(d)                5 max(d)
           0 delta1                0 min(o(q))                0 max(o(q))

22879 cardinality free variables:
       0.077 mean(c(r))              -0 min(c(r))              0.1 max(c(r))
           0 alpha1                0 min(o(r))                0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1      0.50  7.6657e+05    -8.8e+06    -2.2e+04     1.6e+03           0           0           0      -236
   2      0.75       184.22    -8.5e+02    -2.3e+04     1.1e+03           0           0           0      -238
   3      1.12       98.541    -4.8e+02    -2.3e+04     7.3e+02           0           0           0      -239
   4      1.69       61.895    -2.6e+02    -2.3e+04     4.9e+02           0           0           0      -239
   5      2.53       40.001    -1.7e+02    -2.4e+04     3.3e+02           0           0           0      -239
   6      3.80       26.092    -1.3e+02    -2.4e+04     2.2e+02           0           0           0      -239
   7      5.70       18.183         -73    -2.4e+04     1.5e+02           0           0           0      -239
   8      8.54       11.708         -68    -2.4e+04          97           0           0           0      -239
   9     12.81        7.942         -34    -2.4e+04          65           0           0           0      -239
  10     19.22       5.2426         -36    -2.4e+04          44           0           0           0      -239
  11     28.83       3.5411         -48    -2.4e+04          29           0           0           0      -240
  12     43.25       2.3919         -17    -2.4e+04          20           0           0           0      -240
  13     64.87       1.6485         -13    -2.4e+04          14           0           0           0      -240
  14     97.31       1.0952        -4.2    -2.4e+04         9.3           0           0           0      -240
  15    145.96      0.76091        -2.9    -2.4e+04         6.5           0           0           0      -240
  16    218.95      0.51853         -42    -2.4e+04         4.7           0           0           0      -240
  17    328.42      0.53726         -14    -2.4e+04         3.5           0           0           0      -240
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.

cycleFreeFlux: No solution found.
Debugging relaxation etc...
            full: []
             obj: []
           rcost: []
            dual: []
           slack: []
          solver: 'ibm_cplex'
       algorithm: 'Automatic'
            stat: 0
        origStat: 3
    origStatText: 'Model has been proved infeasible'
            time: 0.0300
           basis: []

  itn         obj     obj_old    err(obj)      err(x)   card(v)   card(r)   card(p)   card(q)
    0      14.072       66705       66691      165.16      3913         0       543       541
    1        1391      14.072      1376.9      48.563      2734         0       692       717
    2      944.42        1391      446.54      62.844      3799         0       652       666
    3      1526.3      944.42      581.91      64.636      3026         0       712       702
    4      828.59      1526.3      697.73       63.09      3157         0       574       605
    5      992.49      828.59       163.9      55.391      3334         0       716       682
    6      1074.4      992.49      81.866      59.058      3674         0       687       657
    7      1300.5      1074.4      226.18       63.35      3238         0       700       708
    8      1040.9      1300.5      259.63      62.911      3773         0       685       639
    9      1327.8      1040.9      286.85      63.278      3013         0       703       667
   10      814.59      1327.8      513.18       60.13      3064         0       614       603
   11      1027.5      814.59      212.94      55.715      2717         0       628       614
   12      677.72      1027.5       349.8      53.573      3435         0       602       618
   13      1061.2      677.72      383.44      54.092      3505         0       734       693
   14      1272.7      1061.2      211.54      62.794      3509         0       693       670
   15      1197.8      1272.7      74.901      64.628      3152         0       669       689
   16      971.72      1197.8      226.09      60.517      2983         0       585       622
   17      903.71      971.72      68.006      56.208      2923         0       612       658
   18      834.71      903.71          69      54.078      3806         0       671       642
```

```
   19      1348.3      834.71      513.59      60.702      3075         0       720       659
  itn         obj     obj_old    err(obj)      err(x)   card(v)   card(r)   card(p)   card(q)
Relaxed model is feasible.
Statistics:
599 lower bound relaxation(s)
576 upper bound relaxation(s)
0 steady state relaxation(s)
... done.
ans = 9.1346e-07
ans = 0
ans = 0
solution = struct with fields:
    stat: 1
       v: [19391×1 double]
       r: [23417×1 double]
       p: [19391×1 double]
       q: [19391×1 double]
solutionRelaxed1 = struct with fields:
            full: [19391×1 double]
             obj: 26.3611
           rcost: [19391×1 double]
            dual: [23417×1 double]
           slack: [23417×1 double]
          solver: 'ibm_cplex'
       algorithm: 'Automatic'
            stat: 1
        origStat: 1
    origStatText: 'Optimal solution found'
            time: 1.2415
           basis: []
solutionRelaxed2 = struct with fields:
            full: [19391×1 double]
             obj: 0
           rcost: [19391×1 double]
            dual: [23417×1 double]
           slack: [23417×1 double]
          solver: 'ibm_cplex'
       algorithm: 'Automatic'
            stat: 1
        origStat: 1
    origStatText: 'Optimal solution found'
            time: 0.6092
           basis: []
solutionRelaxed3 = struct with fields:
            full: [19391×1 double]
             obj: 0
           rcost: [19391×1 double]
            dual: [23417×1 double]
           slack: [23417×1 double]
          solver: 'ibm_cplex'
       algorithm: 'Automatic'
            stat: 1
        origStat: 1
    origStatText: 'Optimal solution found'
            time: 0.3797
           basis: []
cycleFreeFlux: No solution found, try using a different solver.

cycleFreeFlux: No solution found.
Debugging relaxation etc...
            full: []
             obj: []
           rcost: []
            dual: []
```

```
          slack: []
         solver: 'ibm_cplex'
      algorithm: 'Automatic'
           stat: 0
       origStat: 3
   origStatText: 'Model has been proved infeasible'
           time: 0.0305
          basis: []
```

itn	obj	obj_old	err(obj)	err(x)	card(v)	card(r)	card(p)	card(q)
0	14.072	66355	66341	164.43	3913	0	543	541
1	1391	14.072	1376.9	48.563	2734	0	692	717
2	944.42	1391	446.54	62.844	3799	0	652	666
3	1526.3	944.42	581.91	64.636	3026	0	712	702
4	828.59	1526.3	697.73	63.09	3157	0	574	605
5	992.49	828.59	163.9	55.391	3334	0	716	682
6	1074.4	992.49	81.866	59.058	3674	0	687	657
7	1300.5	1074.4	226.18	63.35	3238	0	700	708
8	1040.9	1300.5	259.63	62.911	3773	0	685	639
9	1327.8	1040.9	286.85	63.278	3013	0	703	667
10	814.59	1327.8	513.18	60.13	3064	0	614	603
11	1027.5	814.59	212.94	55.715	2717	0	628	614
12	677.72	1027.5	349.8	53.573	3435	0	602	618
13	1061.2	677.72	383.44	54.092	3505	0	734	693
14	1272.7	1061.2	211.54	62.794	3509	0	693	670
15	1197.8	1272.7	74.901	64.628	3152	0	669	689
16	971.72	1197.8	226.09	60.517	2983	0	585	622
17	903.71	971.72	68.006	56.208	2923	0	612	658
18	834.71	903.71	69	54.078	3806	0	671	642
19	1348.3	834.71	513.59	60.702	3075	0	720	659
itn	obj	obj_old	err(obj)	err(x)	card(v)	card(r)	card(p)	card(q)

```
Relaxed model is feasible.
Statistics:
599 lower bound relaxation(s)
576 upper bound relaxation(s)
0 steady state relaxation(s)
... done.
ans = 9.1346e-07
ans = 0
ans = 0
solution = struct with fields:
    stat: 1
       v: [19391×1 double]
       r: [23417×1 double]
       p: [19391×1 double]
       q: [19391×1 double]
solutionRelaxed1 = struct with fields:
           full: [19391×1 double]
            obj: 26.3611
          rcost: [19391×1 double]
           dual: [23417×1 double]
          slack: [23417×1 double]
         solver: 'ibm_cplex'
      algorithm: 'Automatic'
           stat: 1
       origStat: 1
   origStatText: 'Optimal solution found'
           time: 1.3163
          basis: []
solutionRelaxed2 = struct with fields:
           full: [19391×1 double]
            obj: 0
          rcost: [19391×1 double]
           dual: [23417×1 double]
```

```
           slack: [23417×1 double]
          solver: 'ibm_cplex'
       algorithm: 'Automatic'
            stat: 1
         origStat: 1
    origStatText: 'Optimal solution found'
            time: 0.5761
           basis: []
solutionRelaxed3 = struct with fields:
            full: [19391×1 double]
             obj: 0
           rcost: [19391×1 double]
            dual: [23417×1 double]
           slack: [23417×1 double]
          solver: 'ibm_cplex'
       algorithm: 'Automatic'
            stat: 1
         origStat: 1
    origStatText: 'Optimal solution found'
            time: 0.3650
           basis: []
Warning: cycleFreeFlux did not solve, trying v2QNTy
76.81% thermodynamically feasible internal fluxes (checked by v2QNty method).
                iter            card(y)                 nz       %feas int.nz.   tot %feas int.nz.   tot
                   1               5303               7882                0.68                0.50
                   2               2376               3075                1.00                0.69
                   3               1427               1708                1.00                0.77
                   4               1022               1296                1.00                0.83
                   5                769                794                1.00                0.86
                   6                606                587                1.00                0.88
                   7                506                432                1.00                0.90
                   8                449                377                1.00                0.91
                   9                389                324                1.00                0.92
                  10                348                301                1.00                0.93
                  11                298                332                1.00                0.94
                  12                254                384                1.00                0.95
                  13                216                209                1.00                0.96
                  14                191                200                1.00                0.97
                  15                153                201                1.00                0.97
                  16                151                162                1.00                0.97
                  17                106                139                1.00                0.98
                  18                102                161                1.00                0.98
                  19                 78                127                1.00                0.98
                  20                 91                128                1.00                0.98
                iter            card(y)                 nz       %feas int.nz.   tot %feas int.nz.   tot
findThermoConsistentFluxSubset terminating early: n = nMax = 20
--- findThermoFluxConsistentSubset END ----
```

Size of the largest flux, stoich and thermo consistent submodel

```
[nMet,nRxn]=size(stoichFluxThermoConsistModel.S)
save(['~/work/sbgCloud/programModelling/projects/thermoModel/results/
thermoKernel/' modelToLoad
'_stoichFluxThermoConsistModel.mat'],'stoichFluxThermoConsistModel')
```

```
%modelToLoad='Recon3DModel';
load(['~/work/sbgCloud/programModelling/projects/thermoModel/results/
thermoKernel/' modelToLoad
'_stoichFluxThermoConsistModel.mat'],'stoichFluxThermoConsistModel')
```

Nullspace

Nullspace is necessary for backup check of thermodynamic consistency using thermoFlux2QNty

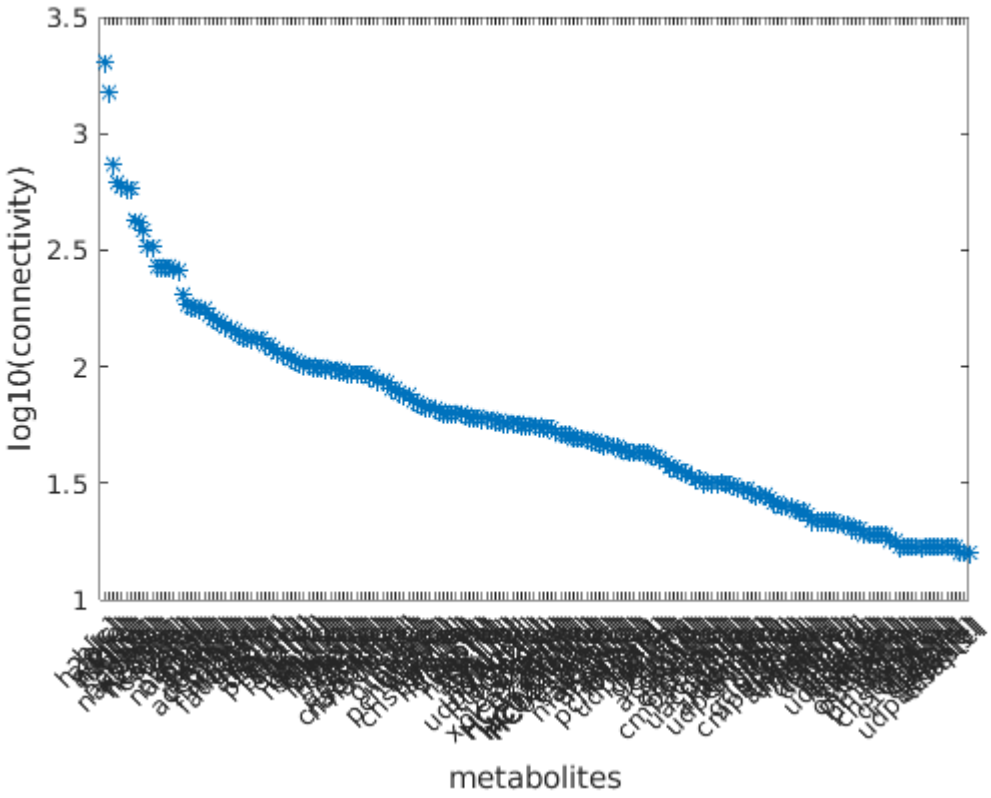
```
[stoichFluxThermoConsistModel,rankK,nnzK,timeTaken] =
internalNullspace(stoichFluxThermoConsistModel);
rankK
```

```
rankK = 5485
```

Data to define a thermodynamically consistent subnetwork

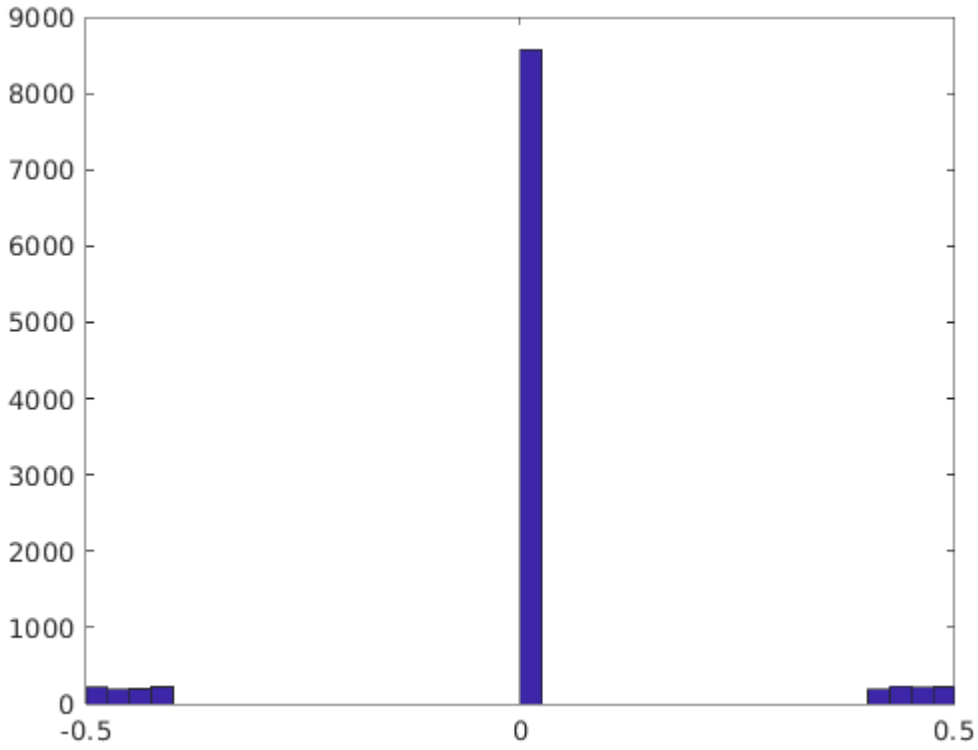
Setup random data to select a random subset

```
param.n=200;
[rankMetConnectivity,rankMetInd,rankConnectivity] =
rankMetabolicConnectivity(stoichFluxThermoConsistModel,param);
```

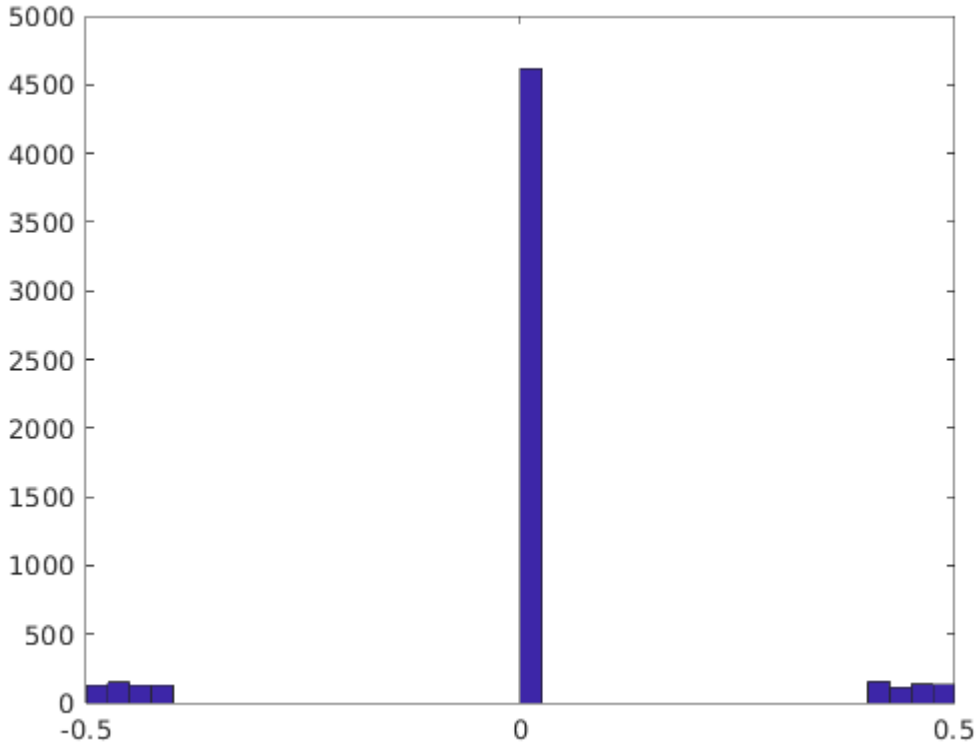



```
[nMet,nRxn]=size(stoichFluxThermoConsistModel.S);
rxnWeights=rand(nRxn,1)-0.5;
rxnWeights(~stoichFluxThermoConsistModel.SConsistentRxnBool)=0;

coreRxnBool=rxnWeights<-0.45;
removeRxnBool=rxnWeights>0.45;
if 0
```

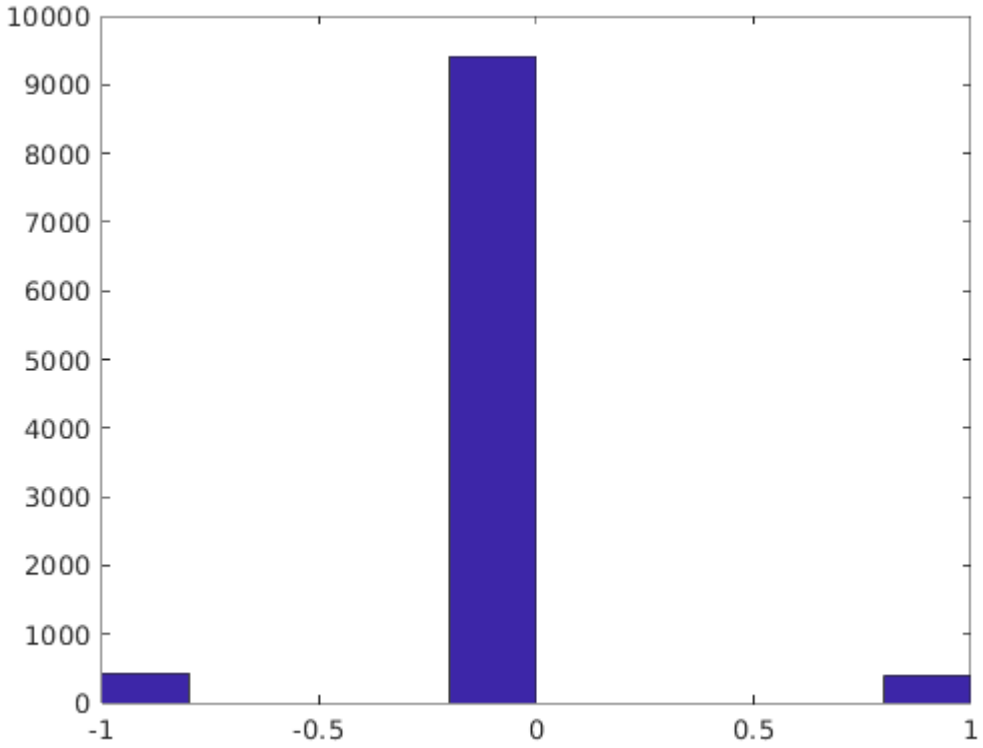
```
    rxnWeights(rxnWeights>0.4)=1;
    rxnWeights(rxnWeights<-0.4)=-1;
end
rxnWeights(rxnWeights>=-0.4 & rxnWeights<=0.4)=0;
hist(rxnWeights,40)
```



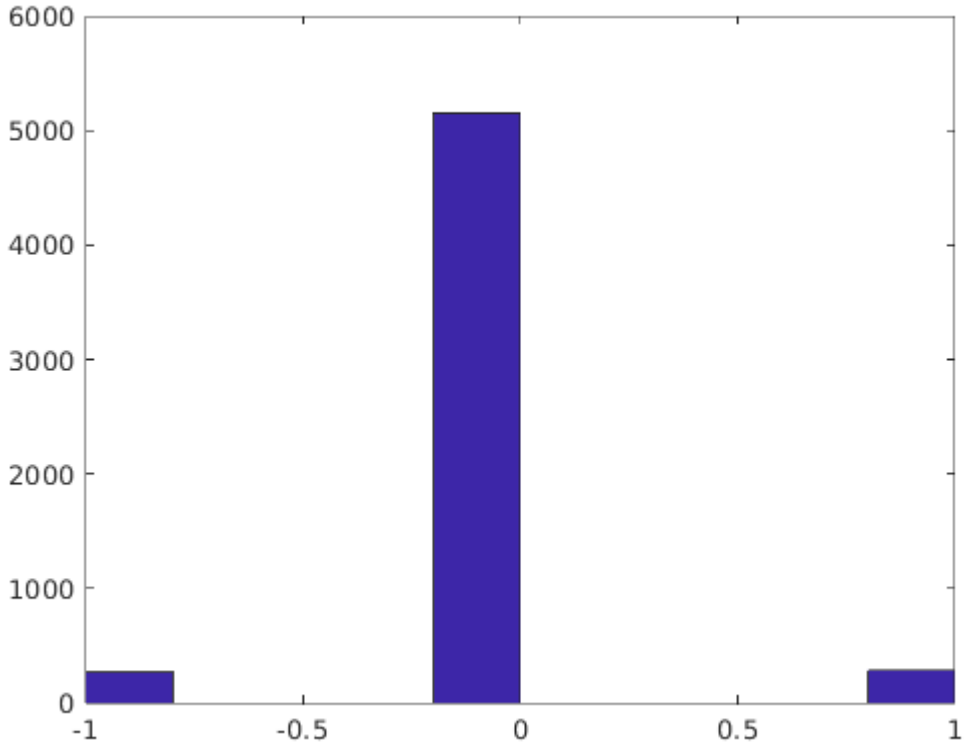

```
metWeights=rand(nMet,1)-0.5;
metWeights(rankMetInd(1:200))=0;
coreMetBool=metWeights<-0.45;
removeMetBool=metWeights>0.45;
if 0
    metWeights(metWeights>0.4)=1;
    metWeights(metWeights<-0.4)=-1;
end
metWeights(metWeights>=-0.4 & metWeights<=0.4)=0;
hist(metWeights,40)
```




```
nlt=length(coreRxnBool);
activeInactiveRxn=zeros(nlt,1);
activeInactiveRxn(coreRxnBool)=1;
activeInactiveRxn(removeRxnBool)=-1;
hist(activeInactiveRxn)
```




```
mlt=length(coreMetBool);
presentAbsentMet=zeros(mlt,1);
presentAbsentMet(coreMetBool)=1;
presentAbsentMet(removeMetBool)=-1;
if 0
    activeInactiveRxn(:)=0;
    presentAbsentMet(:)=0;
end
param.normalizeZeroNormWeights=0;

hist(presentAbsentMet)
```



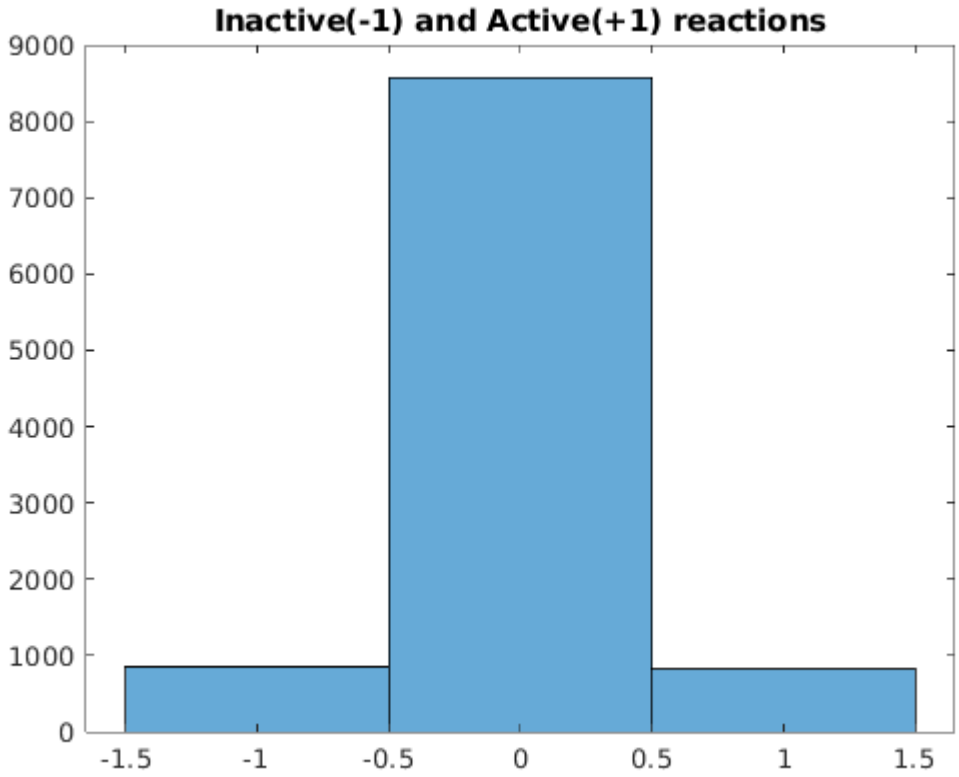
Compute the smallest thermodynamically consistent subnetwork given a list of present/absent metabolites and active/inactive reactions

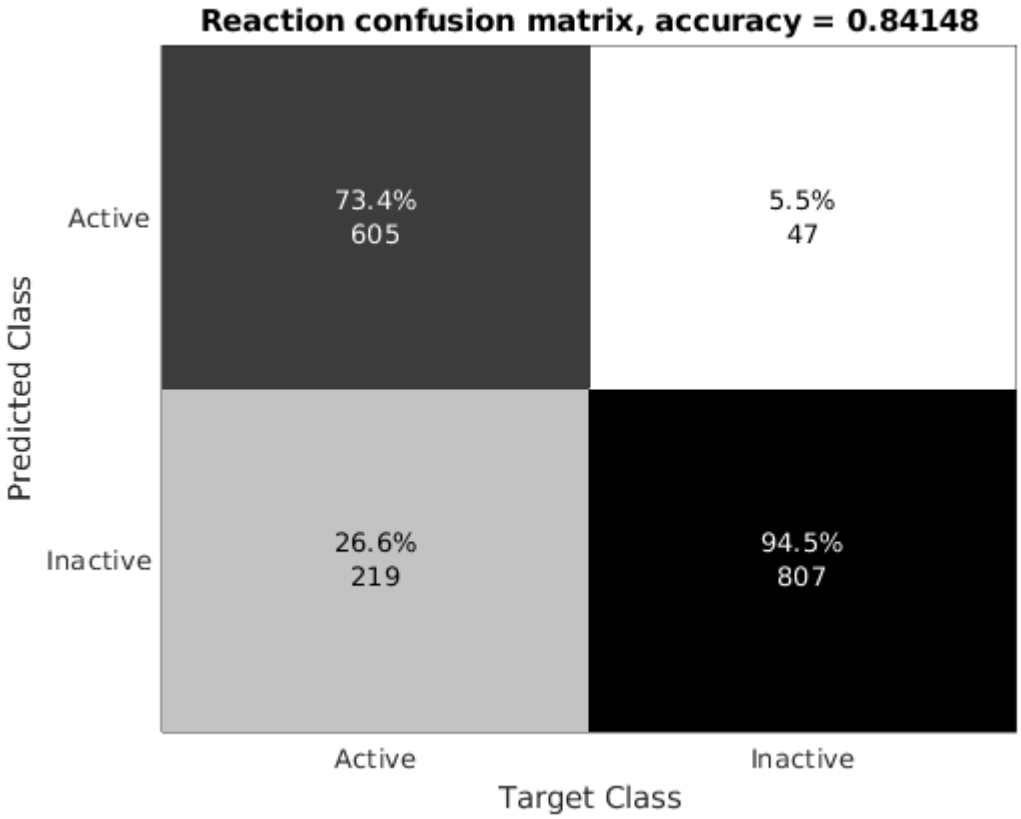
```
[tissueModel, thermoModelMetBool, thermoModelRxnBool] =
thermoKernel(stoichFluxThermoConsistModel, activeInactiveRxn, rxnWeights,
presentAbsentMet, metWeights,param);
```

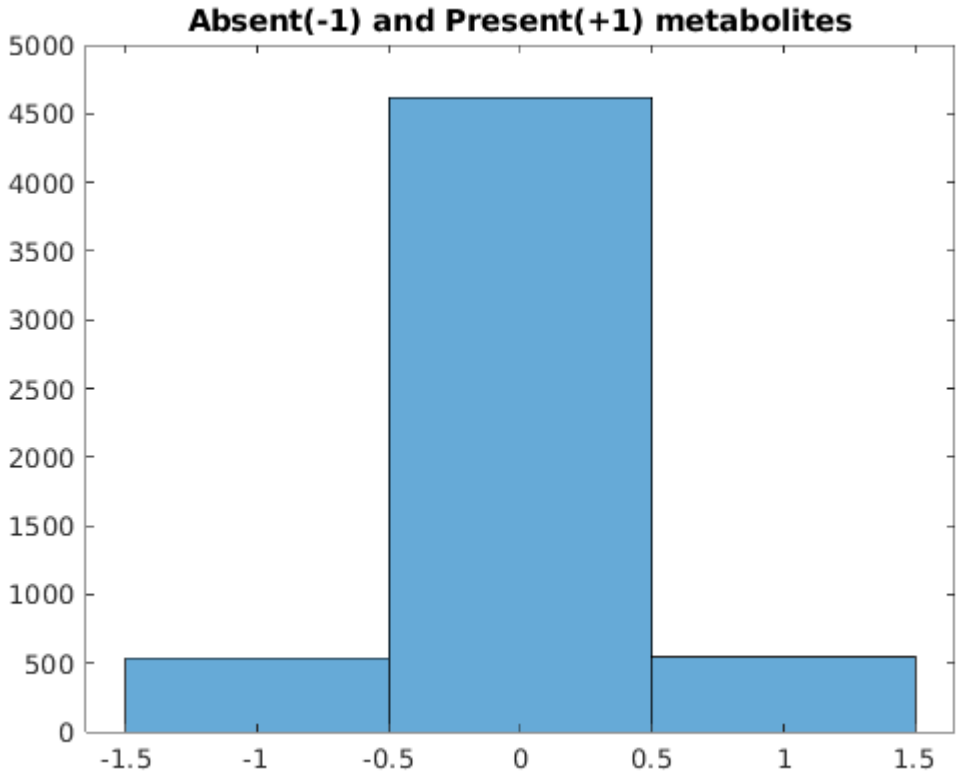
```
--- thermoKernel START ----
thermoKernel parameters:
                  printLevel: 1
                 relaxBounds: 0
           acceptRepairedFlux: 1
                            n: 200
    normalizeZeroNormWeights: 0
                 formulation: 'pqzwrs'
                     epsilon: 1.0000e-06
           removeOrphanGenes: 1
              nbMaxIteration: 30
                        nMax: 20
            iterationMethod: 'greedyRandom'

            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5000
                      theta: 0.5000
            regularizeOuter: 1
                    epsilon: 1.0000e-06
                 printLevel: 1
                relaxBounds: 0
          acceptRepairedFlux: 1
```

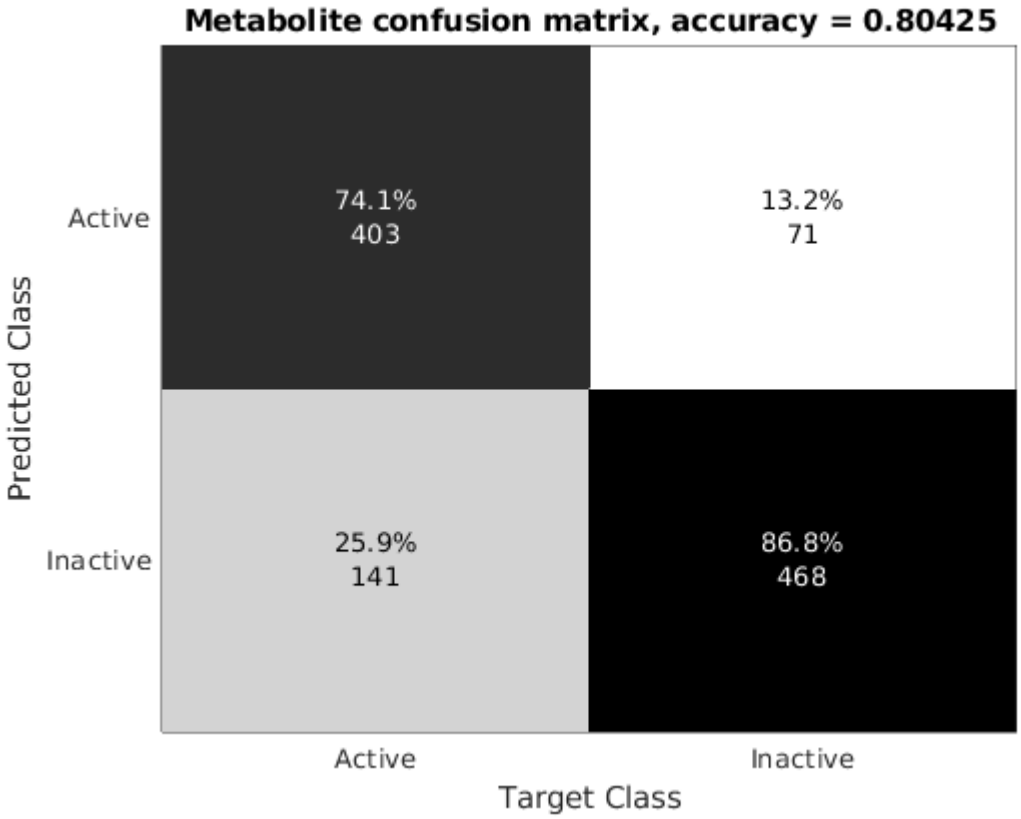
```
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
          1 = beta, the global weight on one-norm of internal reaction rate.
       -0.5 = min(g0), the local weight on zero-norm of internal reaction rate.
        0.5 = max(g0), the local weight on zero-norm of internal reaction rate.
       -0.5 = min(h0), the local weight on zero-norm of metabolite production rate.
        0.5 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

1393 min cardinality variables:
          0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
          1 lambda0             0.4 min(k)              0.5 max(k)
          0 lambda1               0 min(o(p))               0 max(o(p))

1912 max cardinality variables:
          0 mean(c(q))              -0 min(c(q))              -0 max(c(q))
          1 delta0              0.4 min(d)              0.5 max(d)
          0 delta1                0 min(o(q))               0 max(o(q))

35242 cardinality free variables:
       0.48 mean(c(r))              -0 min(c(r))               1 max(c(r))
          0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.6992e+05    -8.5e+07         7.9          11     1.64389        0.11           0    -14.90
   2    0.75      4.5121        -1.7         6.2          12      2.0597        0.16           0    -15.84
   3    1.12      2.8094        -1.8         4.4          12     1.65769        0.25           0    -17.51
   4    1.69      3.0116          -2         2.4          12     1.23553        0.43           0    -18.81
   5    2.53      3.0252        -2.3       0.041          13     1.23553        0.45           0    -19.68
   6    3.80      2.7007        -3.3        -3.3          13     1.23553        0.47           0    -24.78
   7    5.70      2.0612        -4.9        -8.2          14     1.23553        0.87           0    -25.69
   8    8.54      1.9653        -2.8         -11          13     1.23553         0.9           0    -27.03
   9   12.81      1.3137        -3.8         -15          12     1.23553        0.95           0    -30.49
  10   19.22      1.2354          -4         -19          11     1.23553           1           0    -32.32
  11   28.83       0.809        -2.2         -21          10     1.23553         1.1           0    -32.32
  12   43.25     0.71837       -0.57         -21         9.6     1.23553         1.2           0    -32.32
  13   64.87     0.46434       -0.55         -22         9.2     1.23553         1.2           0    -33.26
  14   97.31     0.33824        -1.1         -23         8.9     1.23553         1.2           0    -33.26
  15  145.96     0.21621       -0.14         -23         8.8     1.23553         1.2           0    -33.26
  16  218.95      0.2235      -0.088         -23         8.7     1.23553         1.2           0    -33.26
  17  328.42     0.10113      -0.058         -23         8.6     1.23553         1.2           0    -33.26
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
           warmStartMethod: 'random'
               formulation: 'pqzwrs'
           thetaMultiplier: 1.5000
                     theta: 0.5000
           regularizeOuter: 1
                   epsilon: 1.0000e-06
                printLevel: 1
               relaxBounds: 0
         acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
          1 = beta, the global weight on one-norm of internal reaction rate.
       -0.5 = min(g0), the local weight on zero-norm of internal reaction rate.
```

```
         0.5 = max(g0), the local weight on zero-norm of internal reaction rate.
        -0.5 = min(h0), the local weight on zero-norm of metabolite production rate.
         0.5 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

1393 min cardinality variables:
           0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0             0.4 min(k)             0.5 max(k)
           0 lambda1               0 min(o(p))               0 max(o(p))

1912 max cardinality variables:
           0 mean(c(q))              -0 min(c(q))              -0 max(c(q))
           1 delta0              0.4 min(d)             0.5 max(d)
           0 delta1                0 min(o(q))               0 max(o(q))

35242 cardinality free variables:
        0.48 mean(c(r))              -0 min(c(r))               1 max(c(r))
           0 alpha1                0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1      0.50  9.6862e+05    -8.4e+07         8.1          11     3.74292        0.11           0    -13.29
   2      0.75      2.3033          -1         7.1          10     2.06605        0.16           0    -12.36
   3      1.12      2.5538        -1.1           6          10     2.06605        0.26           0    -13.24
   4      1.69      2.8214        -1.1         4.8          11     1.23553        0.43           0    -14.56
   5      2.53      3.0167        -2.9           2          12     1.23553        0.45           0    -17.11
   6      3.80      2.0716        -2.1      -0.084          12     1.23553        0.47           0    -18.83
   7      5.70      2.5196        -3.8        -3.9          13    0.832228        0.47           0    -20.67
   8      8.54      1.9952        -3.6        -7.5          13    0.832228         0.5           0    -22.59
   9     12.81       1.589          -3         -10          12    0.832228        0.55           0    -23.07
  10     19.22      1.0613        -1.5         -12          10    0.832228        0.62           0    -23.07
  11     28.83     0.69458       -0.65         -13         9.7    0.832228        0.73           0    -23.07
  12     43.25     0.49103       -0.32         -13         9.3    0.832228        0.83           0    -23.07
  13     64.87     0.24192       -0.24         -13           9    0.832228        0.83           0    -23.07
  14     97.31     0.21816       -0.55         -14         8.9    0.832228        0.83           0    -23.47
  15    145.96     0.17636      -0.075         -14         8.8    0.832228        0.83           0    -23.47
  16    218.95    0.097141      -0.048         -14         8.7    0.832228        0.83           0    -23.47
  17    328.42     0.16623      -0.031         -14         8.7    0.832228        0.83           0    -23.47
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
    1       178             1.00             0.02       121             1.29             0.03      gre
    2       208             1.00             0.04       155             1.19             0.10      gre
    3       335             1.00             0.07       249             1.20             0.22      gre
    4       424             1.00             0.11       301             1.28             0.35      gre
    5       393             1.00             0.20       272             1.30             0.42      gre
    6       505             1.00             0.29       354             1.29             0.51      gre
    7       280             1.00             0.34       208             1.28             0.54      gre
    8       378             1.00             0.41       281             1.30             0.58      gre
    9       395             1.00             0.45       291             1.31             0.63      gre
   10       272             1.00             0.50       211             1.28             0.65      gre
   11       281             1.00             0.54       203             1.36             0.67      gre
   12       315             1.00             0.59       228             1.36             0.68      gre
   13       189             1.00             0.61       134             1.29             0.69      gre
   14       358             1.00             0.65       258             1.40             0.70      gre
   15       299             1.00             0.68       218             1.28             0.72      gre
   16       166             1.00             0.69       116             1.30             0.72      gre
   17       275             1.00             0.70       191             1.45             0.73      gre
   18       249             1.00             0.71       175             1.45             0.73      gre
   19       150             1.00             0.72       107             1.38             0.74      gre
   20       235             1.00             0.73       176             1.36             0.74      gre
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
thermoKernel terminating early: n = nMax = 20
```


Inactive(-1) and Active(+1) reactions
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1.5
-1
-0.5
0
0.5
1
1.5


Reaction confusion matrix, accuracy = 0.84148
Active
Inactive
Predicted Class
73.4%
605
5.5%
47
26.6%
219
94.5%
807
Active
Inactive
Target Class





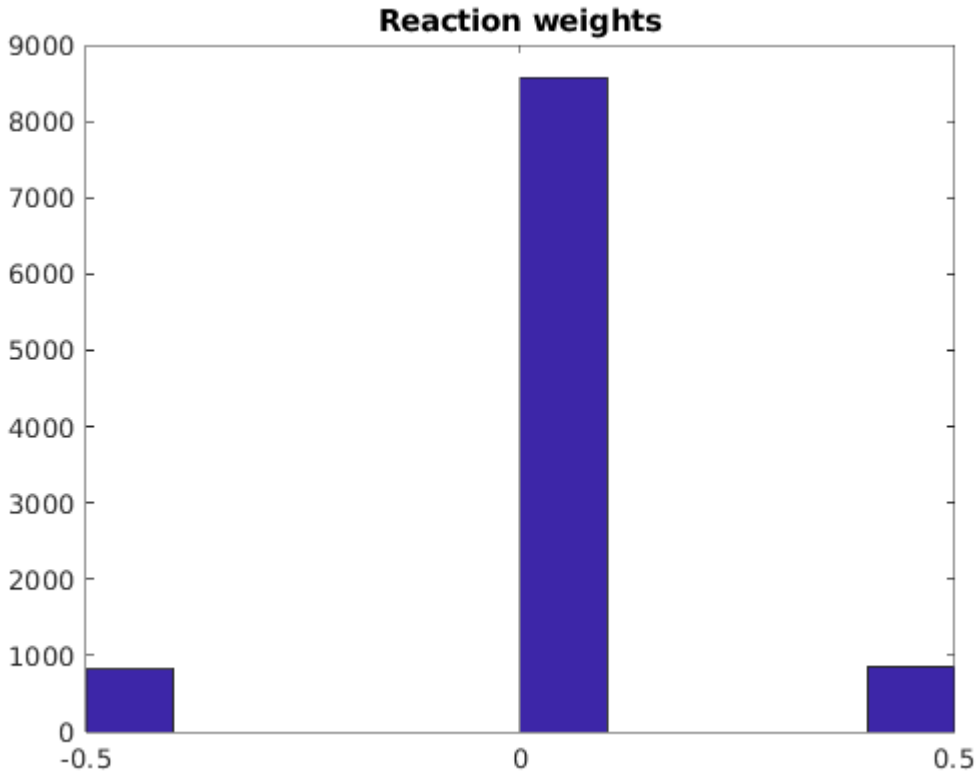

```
--- thermoKernel END ----
```

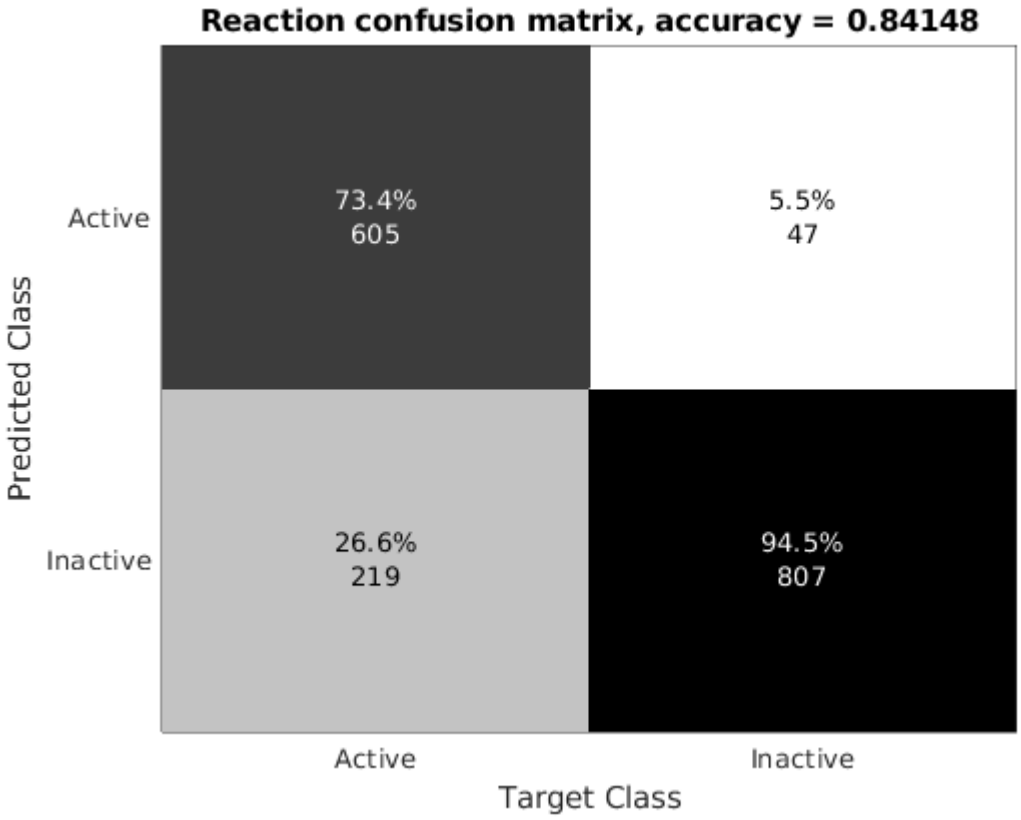
```
[nMet,nRxn]=size(tissueModel.S)
```

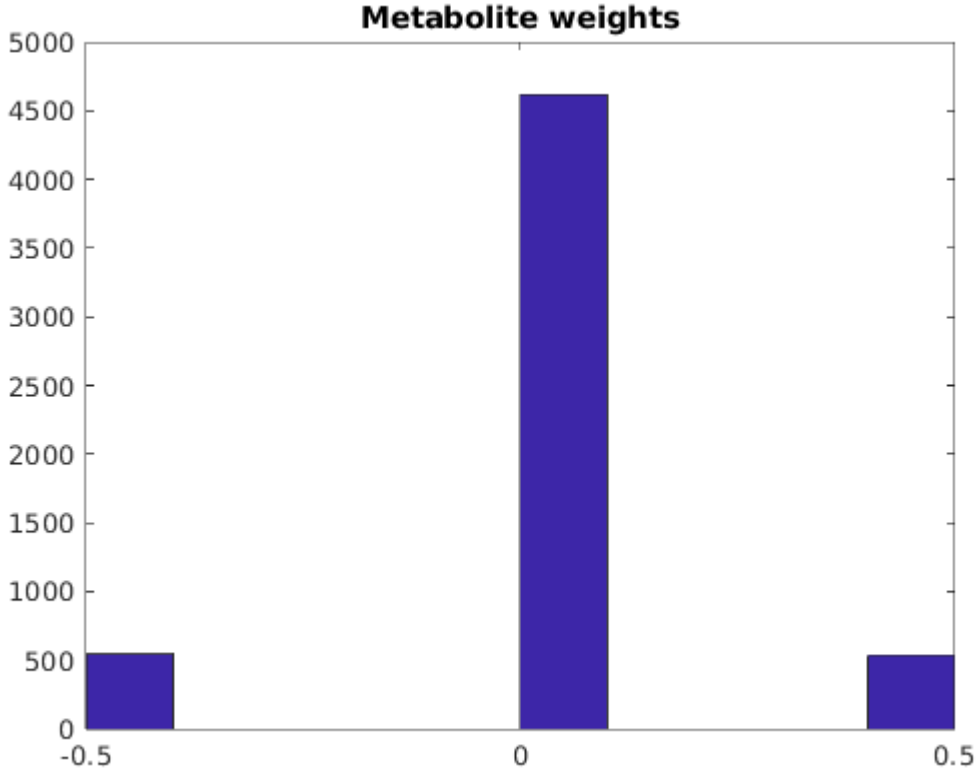
```
nMet = 2203
nRxn = 2986
```

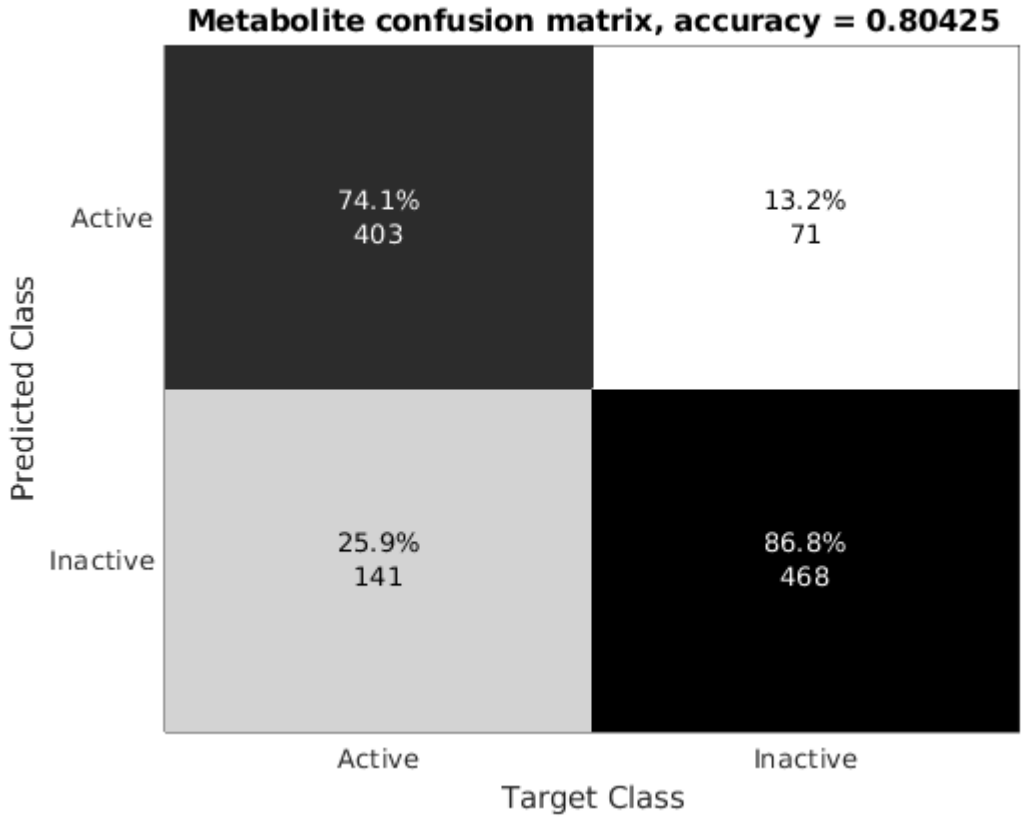
Compare the target versus predicted model

```
%plotThermoCoreStats(activeInactiveRxn, presentAbsentMet,
thermoModelMetBool, thermoModelRxnBool);
plotThermoKernelExtractStats(stoichFluxThermoConsistModel,
activeInactiveRxn, rxnWeights, presentAbsentMet, metWeights,
thermoModelMetBool, thermoModelRxnBool)
```




Reaction confusion matrix, accuracy = 0.84148
Active
Inactive
Predicted Class
73.4%
605
5.5%
47
26.6%
219
94.5%
807
Active
Inactive
Target Class


Metabolite weights
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-0.5
0
0.5



Save weights

```
rxnWeightsTmp=rxnWeights;
metWeightsTmp=metWeights;
```

Submodel with just metabolites specified

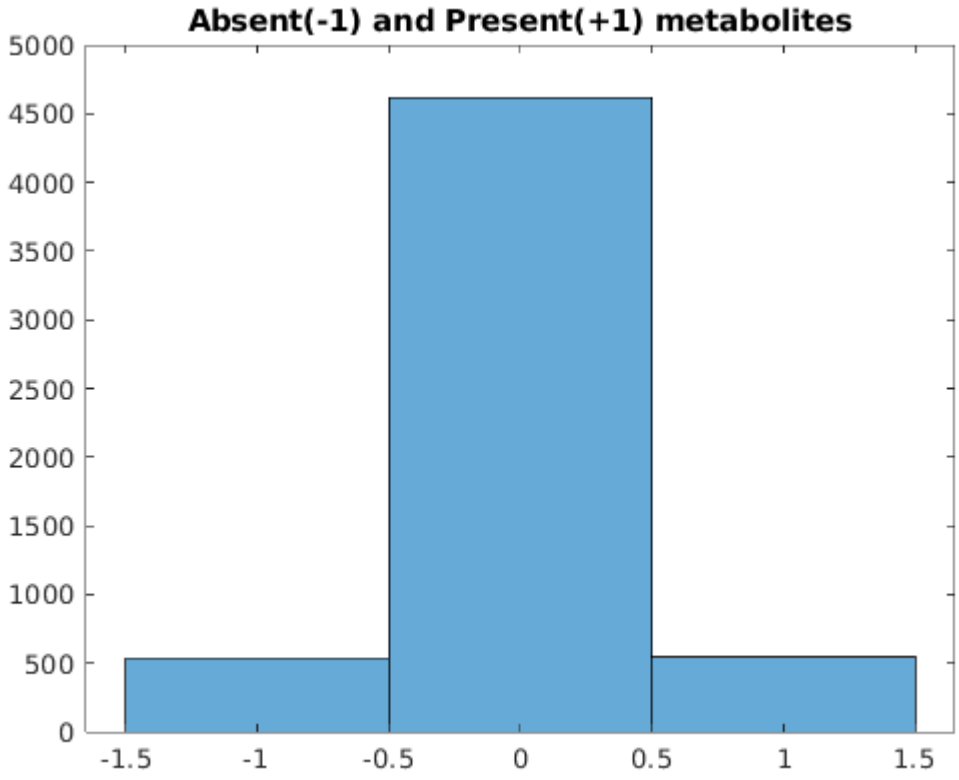
```
metWeights=metWeightsTmp;
rxnWeights(:)=0;
[tissueModel, thermoModelMetBool, thermoModelRxnBool] =
thermoKernel(stoichFluxThermoConsistModel, activeInactiveRxn, rxnWeights,
presentAbsentMet, metWeights,param);
```

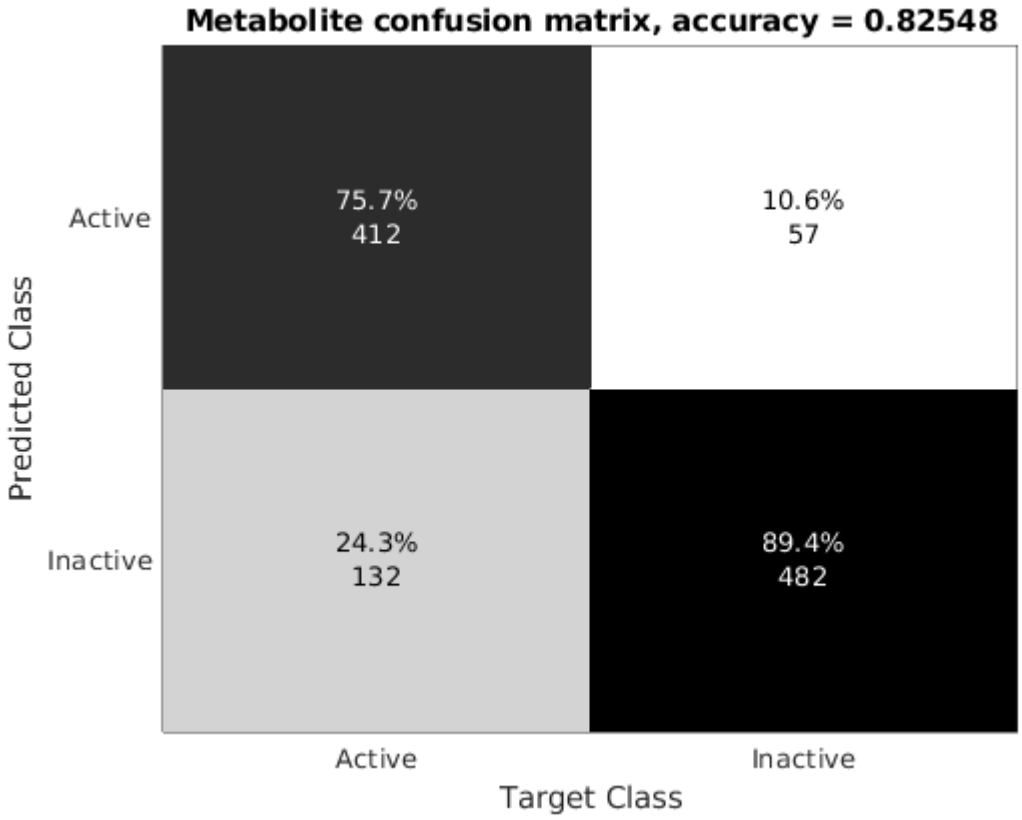
```
--- thermoKernel START ----
thermoKernel parameters:
                  printLevel: 1
                 relaxBounds: 0
          acceptRepairedFlux: 1
                           n: 200
    normalizeZeroNormWeights: 0
                formulation: 'pqzwrs'
                    epsilon: 1.0000e-06
           removeOrphanGenes: 1
              nbMaxIteration: 30
                        nMax: 20
            iterationMethod: 'greedyRandom'

           warmStartMethod: 'random'
```

```
               formulation: 'pqzwrs'
           thetaMultiplier: 1.5000
                     theta: 0.5000
           regularizeOuter: 1
                   epsilon: 1.0000e-06
                printLevel: 1
               relaxBounds: 0
       acceptRepairedFlux: 1
   thermoConsistencyMethod: 'cycleFreeFlux'
                    bigNum: 10000
                     debug: 0

optCardThermo objective data:
          1 = beta, the global weight on one-norm of internal reaction rate.
          0 = min(g0), the local weight on zero-norm of internal reaction rate.
          0 = max(g0), the local weight on zero-norm of internal reaction rate.
       -0.5 = min(h0), the local weight on zero-norm of metabolite production rate.
        0.5 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

539 min cardinality variables:
          0 mean(c(p))              0 min(c(p))              0 max(c(p))
          1 lambda0            0.4 min(k)            0.5 max(k)
          0 lambda1              0 min(o(p))              0 max(o(p))

1088 max cardinality variables:
          0 mean(c(q))              0 min(c(q))              0 max(c(q))
          1 delta0            0.4 min(d)            0.5 max(d)
          0 delta1              0 min(o(q))              0 max(o(q))

36920 cardinality free variables:
       0.46 mean(c(r))             -0 min(c(r))              1 max(c(r))
          0 alpha1              0 min(o(r))              0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.6648e+05    -8.4e+07         7.9          10     2.08529       0.087           0    -7.858
   2    0.75      2.4394       -0.69         7.2         9.4     1.67053        0.13           0    -8.287
   3    1.12       1.334       -0.47         6.7         9.3     1.23553        0.24           0    -8.287
   4    1.69      3.7656          -1         5.7          12     1.23553        0.36           0    -8.287
   5    2.53      3.2162        -1.7           4          11     1.23553        0.45           0    -8.737
   6    3.80      2.1001        -1.8         2.1          11     1.23553        0.47           0    -10.00
   7    5.70      1.3982        -1.2        0.95          10     1.23553        0.51           0    -10.00
   8    8.54     0.78275        -1.5       -0.51         9.8    0.832228         0.5           0      -12.
   9   12.81     0.52353        -1.8        -2.3         9.5    0.832228        0.55           0      -12.
  10   19.22     0.37265       -0.29        -2.6         9.1    0.832228        0.62           0      -12.
  11   28.83     0.33297       -0.11        -2.7         8.9    0.832228        0.73           0      -12.
  12   43.25     0.19468        -0.5        -3.2         8.7    0.832228        0.83           0    -13.21
  13   64.87    0.099656       -0.91        -4.1         8.7    0.832228        0.83           0    -15.03
  14   97.31     0.11652        -1.5        -5.6         8.6    0.832228        0.83           0    -15.03
  15  145.96     0.12539       -0.03        -5.6         8.6    0.832228        0.83           0    -15.03
  16  218.95    0.067216      -0.018        -5.6         8.6    0.832228        0.83           0    -15.03
  17  328.42     0.13964      -0.012        -5.6         8.6    0.832228        0.83           0    -15.03
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
           warmStartMethod: 'random'
               formulation: 'pqzwrs'
           thetaMultiplier: 1.5000
                     theta: 0.5000
           regularizeOuter: 1
                   epsilon: 1.0000e-06
                printLevel: 1
               relaxBounds: 0
```

```
         acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           0 = min(g0), the local weight on zero-norm of internal reaction rate.
           0 = max(g0), the local weight on zero-norm of internal reaction rate.
        -0.5 = min(h0), the local weight on zero-norm of metabolite production rate.
         0.5 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

539 min cardinality variables:
           0 mean(c(p))              0 min(c(p))              0 max(c(p))
           1 lambda0             0.4 min(k)             0.5 max(k)
           0 lambda1               0 min(o(p))              0 max(o(p))

1088 max cardinality variables:
           0 mean(c(q))              0 min(c(q))              0 max(c(q))
           1 delta0              0.4 min(d)             0.5 max(d)
           0 delta1                0 min(o(q))              0 max(o(q))

36920 cardinality free variables:
        0.46 mean(c(r))             -0 min(c(r))              1 max(c(r))
           0 alpha1               0 min(o(r))              0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1      0.50  9.7116e+05    -8.5e+07         7.9          10     2.08529       0.079           0    -7.121
   2      0.75      2.4188       -0.68         7.2         9.5     1.23553        0.11           0    -7.551
   3      1.12      2.6941       -0.72         6.5          10     1.23553        0.24           0    -8.353
   4      1.69      4.9777       -0.99         5.5          11     1.23553        0.36           0    -8.353
   5      2.53      4.4276        -1.7         3.8          11     1.23553        0.45           0    -8.802
   6      3.80      2.3513        -2.2         1.6          11     1.23553        0.47           0    -10.52
   7      5.70      1.6188        -1.2         0.4          10     1.23553        0.51           0    -10.52
   8      8.54     0.63996       -0.59       -0.19         9.6     1.23553        0.56           0    -10.52
   9     12.81     0.55001        -0.9        -1.1         9.4    0.832228        0.55           0    -12.35
  10     19.22      0.4508        -1.5        -2.6         9.1    0.832228        0.62           0    -12.35
  11     28.83     0.42169       -0.11        -2.7         8.9    0.832228        0.73           0    -12.35
  12     43.25     0.24485      -0.032        -2.8         8.7    0.832228        0.83           0    -12.35
  13     64.87     0.19244      -0.075        -2.9         8.7    0.832228        0.83           0    -12.35
  14     97.31     0.10717      -0.042        -2.9         8.6    0.832228        0.83           0    -12.35
  15    145.96    0.083129      -0.023        -2.9         8.6    0.832228        0.83           0    -12.35
  16    218.95    0.070749      -0.015        -2.9         8.6    0.832228        0.83           0    -12.35
  17    328.42     0.14835     -0.0098        -2.9         8.6    0.832228        0.83           0    -12.35
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
    1       137             1.00              NaN        95             1.21             0.03      gre
    2       186             1.00              NaN       139             1.17             0.08      gre
    3       297             1.00              NaN       226             1.19             0.20      gre
    4       467             1.00              NaN       349             1.30             0.37      gre
    5       203             1.00              NaN       149             1.23             0.43      gre
    6       282             1.00              NaN       213             1.24             0.51      gre
    7       285             1.00              NaN       201             1.32             0.56      gre
    8       213             1.00              NaN       156             1.30             0.60      gre
    9       168             1.00              NaN       124             1.27             0.62      gre
   10       132             1.00              NaN        97             1.20             0.63      gre
   11       248             1.00              NaN       184             1.33             0.66      gre
   12       111             1.00              NaN        78             1.22             0.66      gre
   13       200             1.00              NaN       148             1.36             0.68      gre
   14       112             1.00              NaN        77             1.25             0.68      gre
```

```
   15       984              1.00               NaN       709              1.26              0.70
   16      1076              1.00               NaN       789              1.24              0.74
   17      1024              1.00               NaN       744              1.27              0.74
   18      1000              1.00               NaN       726              1.26              0.75
   19       974              1.00               NaN       732              1.22              0.75
   20       965              1.00               NaN       718              1.24              0.76
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
thermoKernel terminating early: n = nMax = 20
```





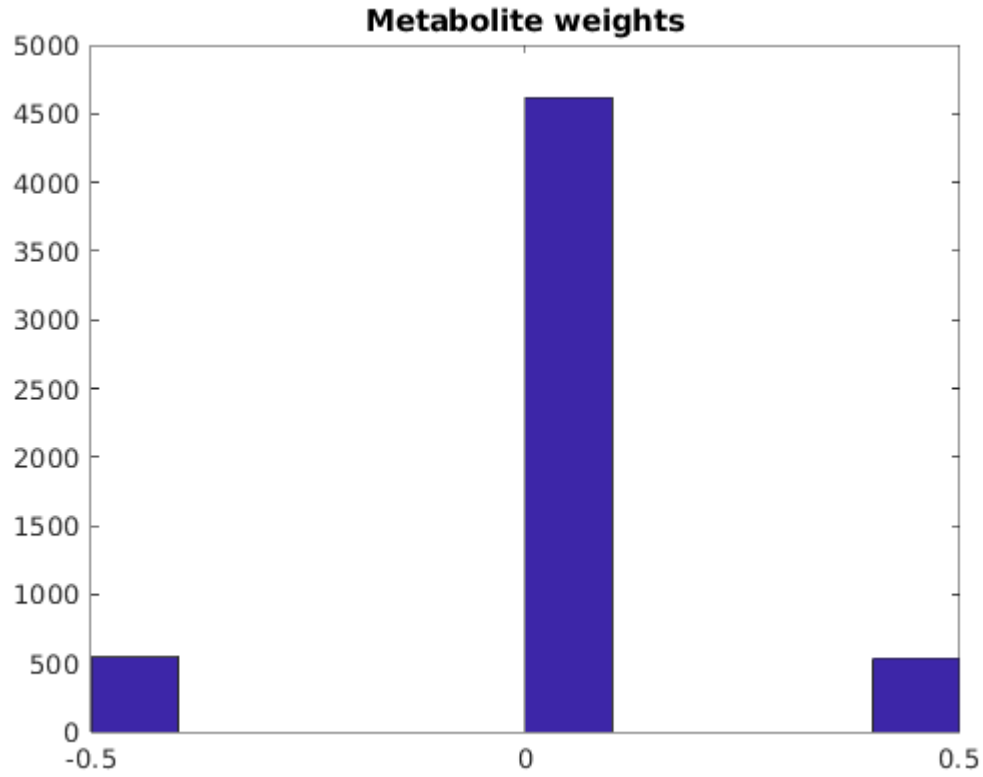

```
--- thermoKernel END ----
```

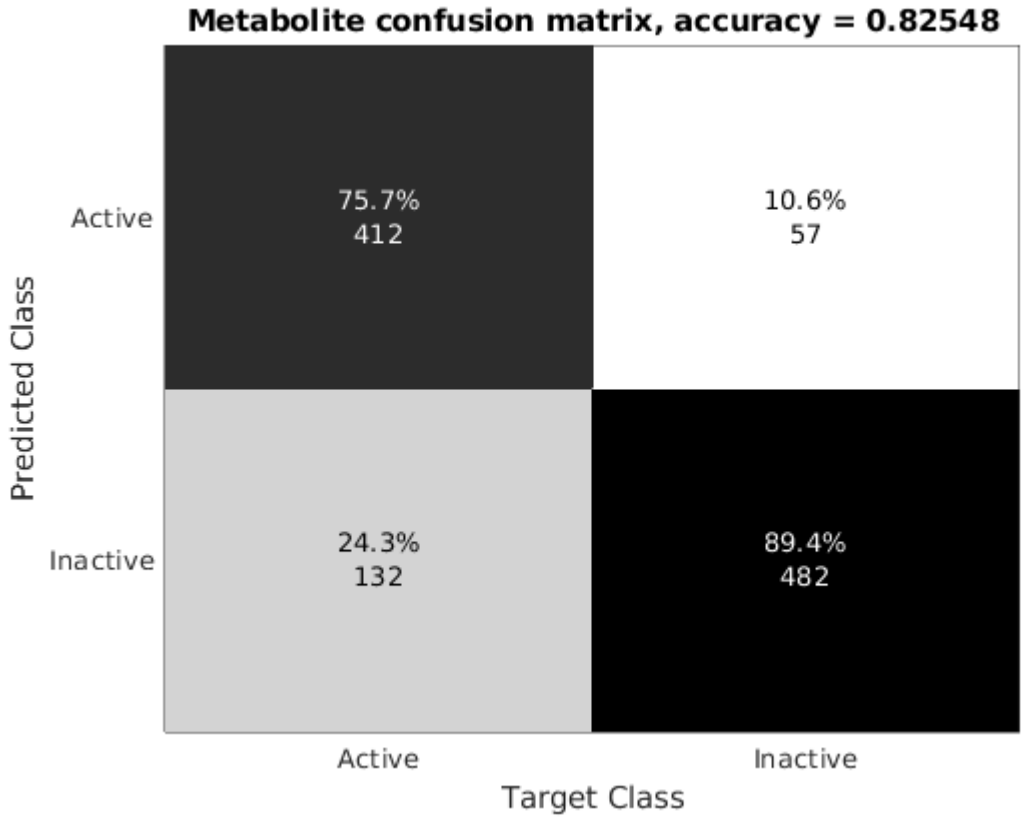
```
[nMet,nRxn]=size(tissueModel.S)
```

```
nMet = 1870
nRxn = 2635
```

Compare the target versus predicted model

```
plotThermoKernelExtractStats(stoichFluxThermoConsistModel,
activeInactiveRxn, rxnWeights, presentAbsentMet, metWeights,
thermoModelMetBool, thermoModelRxnBool)
```





Submodel with just reactions specified

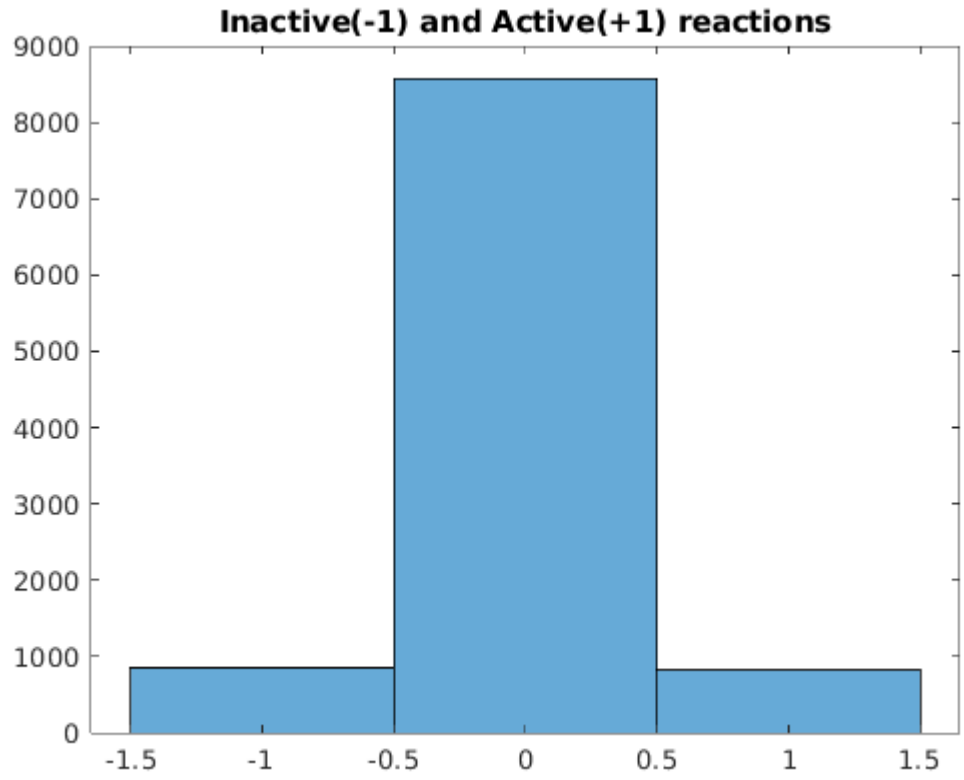
```
rxnWeights=rxnWeightsTmp;
```

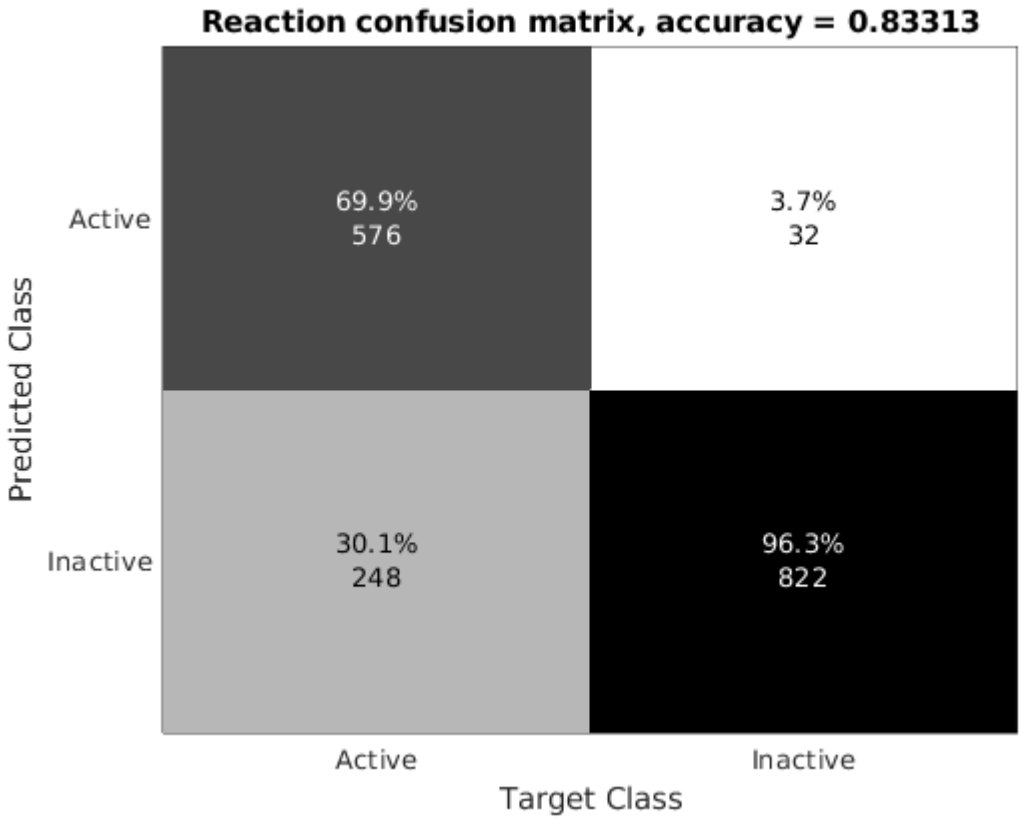
```
metWeights(:)=0;
[tissueModel, thermoModelMetBool, thermoModelRxnBool] =
thermoKernel(stoichFluxThermoConsistModel, activeInactiveRxn, rxnWeights,
presentAbsentMet, metWeights,param);
```

```
--- thermoKernel START ----
thermoKernel parameters:
                  printLevel: 1
                 relaxBounds: 0
           acceptRepairedFlux: 1
                           n: 200
    normalizeZeroNormWeights: 0
                formulation: 'pqzwrs'
                    epsilon: 1.0000e-06
           removeOrphanGenes: 1
              nbMaxIteration: 30
                        nMax: 20
            iterationMethod: 'greedyRandom'

            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5000
                      theta: 0.5000
            regularizeOuter: 1
                    epsilon: 1.0000e-06
                  printLevel: 1
                 relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
        -0.5 = min(g0), the local weight on zero-norm of internal reaction rate.
         0.5 = max(g0), the local weight on zero-norm of internal reaction rate.
           0 = min(h0), the local weight on zero-norm of metabolite production rate.
           0 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

854 min cardinality variables:
          -0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0             0.4 min(k)             0.5 max(k)
           0 lambda1               0 min(o(p))               0 max(o(p))

824 max cardinality variables:
          -0 mean(c(q))              -0 min(c(q))              -0 max(c(q))
           1 delta0             0.4 min(d)             0.5 max(d)
           0 delta1               0 min(o(q))               0 max(o(q))

36869 cardinality free variables:
        0.46 mean(c(r))              -0 min(c(r))               1 max(c(r))
           0 alpha1              0 min(o(r))              0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1      0.50  9.6945e+05    -8.5e+07         8.4         8.4    0.408362        0.02           0    -3.084
   2      0.75    0.082294      -0.032         8.4         8.4    0.408362       0.028           0    -3.506
   3      1.12     0.25722      -0.046         8.3         8.5    0.408362        0.03           0    -3.506
   4      1.69     0.41187      -0.093         8.2         8.5    0.422161      0.0076           0    -3.506
   5      2.53  5.4552e-14       -0.15         8.1         8.5    0.422161       0.011           0    -3.506
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
```

```
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5000
                      theta: 0.5000
            regularizeOuter: 1
                    epsilon: 1.0000e-06
                 printLevel: 1
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
        -0.5 = min(g0), the local weight on zero-norm of internal reaction rate.
         0.5 = max(g0), the local weight on zero-norm of internal reaction rate.
           0 = min(h0), the local weight on zero-norm of metabolite production rate.
           0 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

854 min cardinality variables:
          -0 mean(c(p))              -0 min(c(p))              -0 max(c(p))
           1 lambda0             0.4 min(k)              0.5 max(k)
           0 lambda1               0 min(o(p))               0 max(o(p))

824 max cardinality variables:
          -0 mean(c(q))              -0 min(c(q))              -0 max(c(q))
           1 delta0              0.4 min(d)              0.5 max(d)
           0 delta1                0 min(o(q))               0 max(o(q))

36869 cardinality free variables:
        0.46 mean(c(r))              -0 min(c(r))               1 max(c(r))
           0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.6798e+05    -8.5e+07         8.4         8.4    0.830523        0.02           0    -3.506
   2    0.75     0.11192      -0.032         8.4         8.4    0.408362       0.028           0    -3.506
   3    1.12     0.25722      -0.046         8.3         8.5    0.408362        0.03           0    -3.506
   4    1.69     0.41187      -0.093         8.2         8.5    0.422161      0.0076           0    -3.506
   5    2.53  5.4552e-14       -0.15         8.1         8.5    0.422161       0.011           0    -3.506
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
    1       100             1.00             0.01        72             1.15              NaN      gre
    2        99             1.00             0.01        70             1.21              NaN      gre
    3       113             1.00             0.02        79             1.15              NaN      gre
    4       113             1.00             0.03        83             1.12              NaN      gre
    5       181             1.00             0.07       134             1.14              NaN      gre
    6       240             1.00             0.14       183             1.14              NaN      gre
    7       192             1.00             0.20       128             1.38              NaN      gre
    8       184             1.00             0.24       130             1.31              NaN      gre
    9       276             1.00             0.30       192             1.38              NaN      gre
   10       267             1.00             0.35       193             1.28              NaN      gre
   11       309             1.00             0.42       227             1.29              NaN      gre
   12       293             1.00             0.49       209             1.44              NaN      gre
   13       295             1.00             0.54       205             1.39              NaN      gre
   14       214             1.00             0.57       173             1.25              NaN      gre
   15       340             1.00             0.62       249             1.37              NaN      gre
   16       284             1.00             0.66       203             1.37              NaN      gre
   17       177             1.00             0.67       122             1.42              NaN      gre
```

```
   18       116              1.00              0.67        87              1.20               NaN      gre
   19       147              1.00              0.68       101              1.40               NaN      gre
   20       199              1.00              0.70       139              1.42               NaN      gre
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
thermoKernel terminating early: n = nMax = 20
```





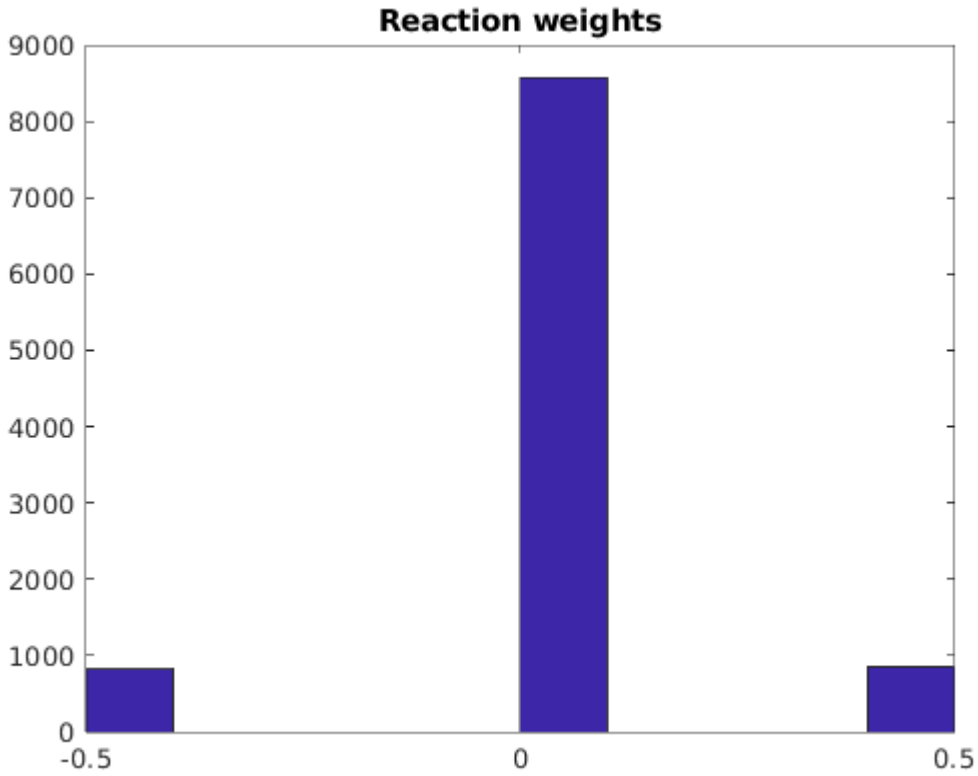
```
--- thermoKernel END ----
```

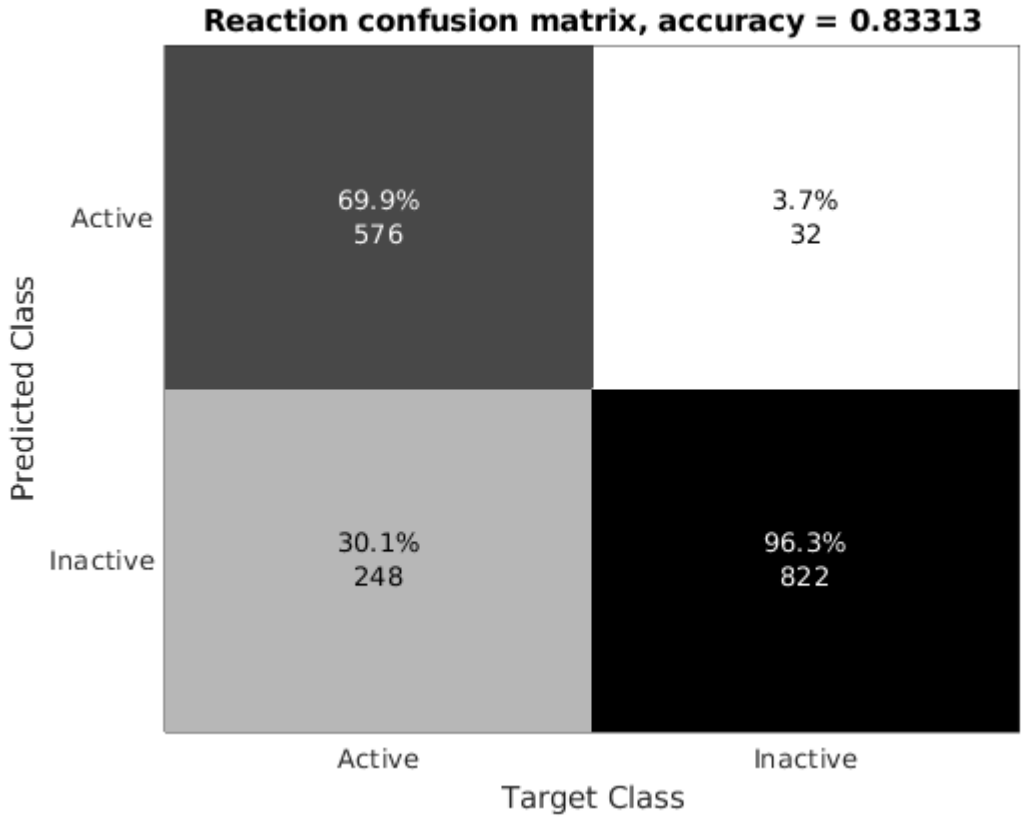
```
[nMet,nRxn]=size(tissueModel.S)
```

```
nMet = 1441
nRxn = 1924
```

Compare the target versus predicted model

```
plotThermoKernelExtractStats(stoichFluxThermoConsistModel,
activeInactiveRxn, rxnWeights, presentAbsentMet, metWeights,
thermoModelMetBool, thermoModelRxnBool)
```





Submodel with just active metabolites specified

```
metWeightsRed=metWeightsTmp;
rxnWeightsRed=rxnWeightsTmp*0;
metWeightsRed(metWeightsRed>=0)=0;
```

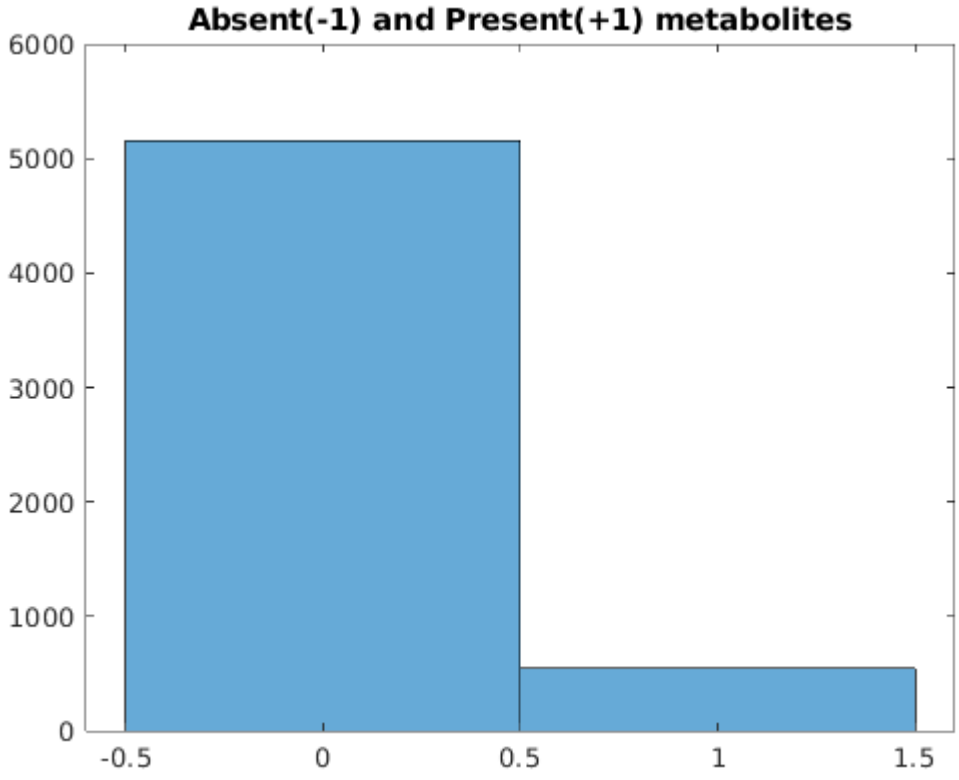
```
[tissueModel, thermoModelMetBool, thermoModelRxnBool] =
thermoKernel(stoichFluxThermoConsistModel, activeInactiveRxn, rxnWeightsRed,
presentAbsentMet, metWeightsRed,param);
```

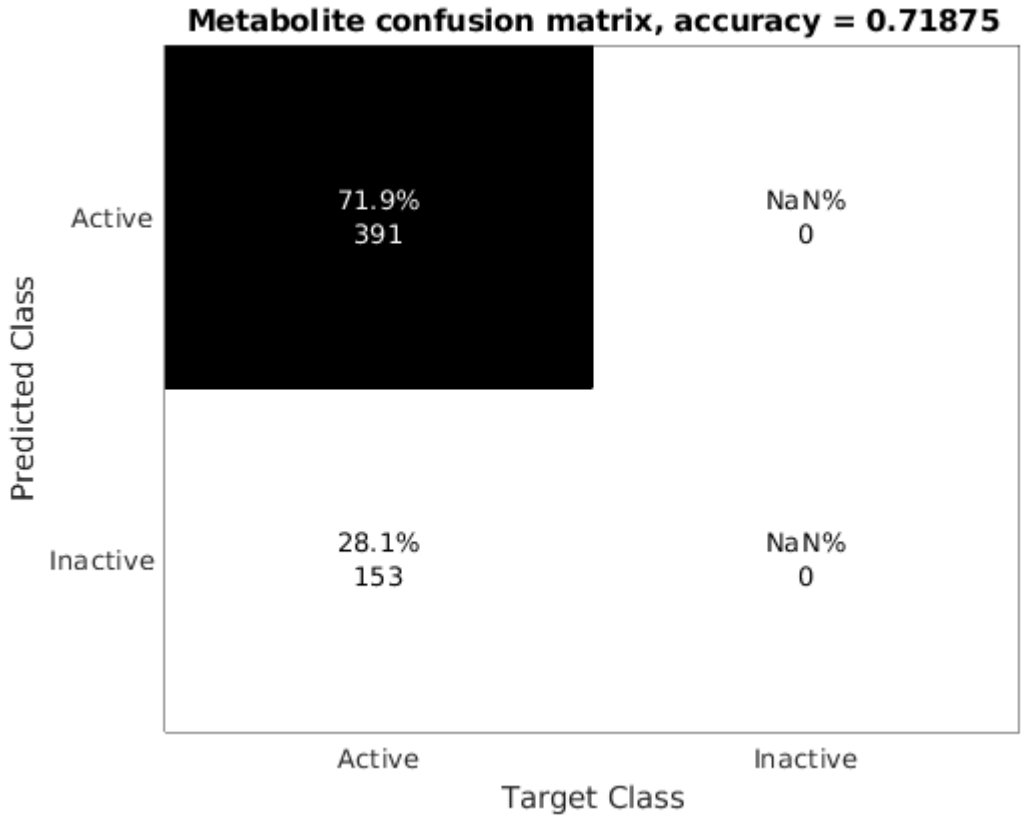
```
--- thermoKernel START ----
thermoKernel parameters:
                  printLevel: 1
                 relaxBounds: 0
           acceptRepairedFlux: 1
                            n: 200
    normalizeZeroNormWeights: 0
                formulation: 'pqzwrs'
                    epsilon: 1.0000e-06
           removeOrphanGenes: 1
              nbMaxIteration: 30
                        nMax: 20
            iterationMethod: 'greedyRandom'

            warmStartMethod: 'random'
                formulation: 'pqzwrs'
```

```
            thetaMultiplier: 1.5000
                      theta: 0.5000
            regularizeOuter: 1
                    epsilon: 1.0000e-06
                 printLevel: 1
                relaxBounds: 0
          acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           0 = min(g0), the local weight on zero-norm of internal reaction rate.
           0 = max(g0), the local weight on zero-norm of internal reaction rate.
        -0.5 = min(h0), the local weight on zero-norm of metabolite production rate.
           0 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

0 min cardinality variables:
         NaN mean(c(p))             NaN min(c(p))             NaN max(c(p))
           1 lambda0             NaN min(k)              NaN max(k)
           0 lambda1             NaN min(o(p))              NaN max(o(p))

1088 max cardinality variables:
           0 mean(c(q))               0 min(c(q))               0 max(c(q))
           1 delta0             0.4 min(d)              0.5 max(d)
           0 delta1               0 min(o(q))               0 max(o(q))

37459 cardinality free variables:
        0.45 mean(c(r))              -0 min(c(r))               1 max(c(r))
           0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.6587e+05    -8.5e+07         7.4          13           0           0           0    -14.77
   2    0.75      5.8717        -2.6         4.8          14           0           0           0    -14.32
   3    1.12      3.0027        -2.1         2.7          13           0           0           0    -15.21
   4    1.69      5.6907        -2.2        0.55          13           0           0           0    -17.00
   5    2.53      5.2054        -4.5        -3.9          14           0           0           0    -20.17
   6    3.80       2.462        -3.4        -7.3          13           0           0           0    -21.59
   7    5.70       1.606        -2.7         -10          12           0           0           0     -22.5
   8    8.54      1.0881        -1.7         -12          11           0           0           0     -22.5
   9   12.81     0.80985       -0.89         -13          10           0           0           0     -22.5
  10   19.22     0.49523       -0.58         -13         9.4           0           0           0     -22.5
  11   28.83     0.38668       -0.36         -13           9           0           0           0     -22.5
  12   43.25     0.28276       -0.38         -14         8.8           0           0           0    -23.47
  13   64.87     0.21412       -0.94         -15         8.7           0           0           0    -23.47
  14   97.31     0.16629      -0.083         -15         8.6           0           0           0    -23.47
  15  145.96    0.084169       -0.26         -15         8.5           0           0           0    -25.28
  16  218.95     0.12117        -1.6         -17         8.5           0           0           0    -25.28
  17  328.42     0.16767       -0.14         -17         8.5           0           0           0    -25.74
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5000
                      theta: 0.5000
            regularizeOuter: 1
                    epsilon: 1.0000e-06
                 printLevel: 1
                relaxBounds: 0
          acceptRepairedFlux: 1
```

```
    thermoConsistencyMethod: 'cycleFreeFlux'
                     bigNum: 10000
                      debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
           0 = min(g0), the local weight on zero-norm of internal reaction rate.
           0 = max(g0), the local weight on zero-norm of internal reaction rate.
        -0.5 = min(h0), the local weight on zero-norm of metabolite production rate.
           0 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

0 min cardinality variables:
         NaN mean(c(p))             NaN min(c(p))             NaN max(c(p))
           1 lambda0             NaN min(k)             NaN max(k)
           0 lambda1             NaN min(o(p))             NaN max(o(p))

1088 max cardinality variables:
           0 mean(c(q))               0 min(c(q))               0 max(c(q))
           1 delta0             0.4 min(d)             0.5 max(d)
           0 delta1               0 min(o(q))               0 max(o(q))

37459 cardinality free variables:
        0.45 mean(c(r))              -0 min(c(r))               1 max(c(r))
           0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.6895e+05    -8.5e+07         8.1           9           0           0           0    -6.131
   2    0.75       1.691       -0.42         7.7         8.8           0           0           0    -6.561
   3    1.12      2.8196       -0.42         7.3          10           0           0           0    -6.561
   4    1.69      3.3125       -0.91         6.4          11           0           0           0    -6.561
   5    2.53      3.3113        -2.3         4.1          11           0           0           0    -7.845
   6    3.80       1.408          -1           3          11           0           0           0    -8.276
   7    5.70      1.4232        -1.5         1.5          10           0           0           0    -8.728
   8    8.54     0.75486       -0.82        0.71         9.6           0           0           0    -9.635
   9   12.81      0.5787        -1.1       -0.43         9.2           0           0           0    -9.635
  10   19.22     0.45877       -0.29       -0.72         8.9           0           0           0    -9.635
  11   28.83     0.19759       -0.19       -0.91         8.7           0           0           0    -9.635
  12   43.25     0.17445       -0.12          -1         8.6           0           0           0    -9.635
  13   64.87      0.1615       -0.44        -1.5         8.5           0           0           0    -11.45
  14   97.31     0.14097        -1.5        -2.9         8.5           0           0           0    -11.45
  15  145.96     0.12588      -0.026          -3         8.5           0           0           0    -11.45
  16  218.95    0.086138      -0.015          -3         8.5           0           0           0    -11.45
  17  328.42    0.074785       -0.01          -3         8.5           0           0           0    -11.45
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
    1       122             1.00              NaN        86             1.16              0.02      gre
    2       230             1.00              NaN       172             1.16              0.10      gre
    3       231             1.00              NaN       183             1.18              0.18      gre
    4       352             1.00              NaN       263             1.22              0.31      gre
    5       283             1.00              NaN       206             1.32              0.40      gre
    6       274             1.00              NaN       199             1.37              0.47      gre
    7       275             1.00              NaN       201             1.34              0.54      gre
    8       175             1.00              NaN       139             1.19              0.56      gre
    9       130             1.00              NaN        97             1.20              0.58      gre
   10       307             1.00              NaN       235             1.34              0.62      gre
   11       243             1.00              NaN       183             1.31              0.65      gre
   12       120             1.00              NaN        84             1.29              0.65      gre
   13       218             1.00              NaN       158             1.32              0.67      gre
   14       113             1.00              NaN        80             1.24              0.67      gre
   15       131             1.00              NaN        99             1.30              0.68      gre
```

```
   16       129              1.00               NaN        92              1.30              0.69      gre
   17       164              1.00               NaN       116              1.30              0.69      gre
   18       166              1.00               NaN       123              1.26              0.70      gre
   19       165              1.00               NaN       117              1.35              0.71      gre
   20       141              1.00               NaN        95              1.36              0.72      gre
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
thermoKernel terminating early: n = nMax = 20
```





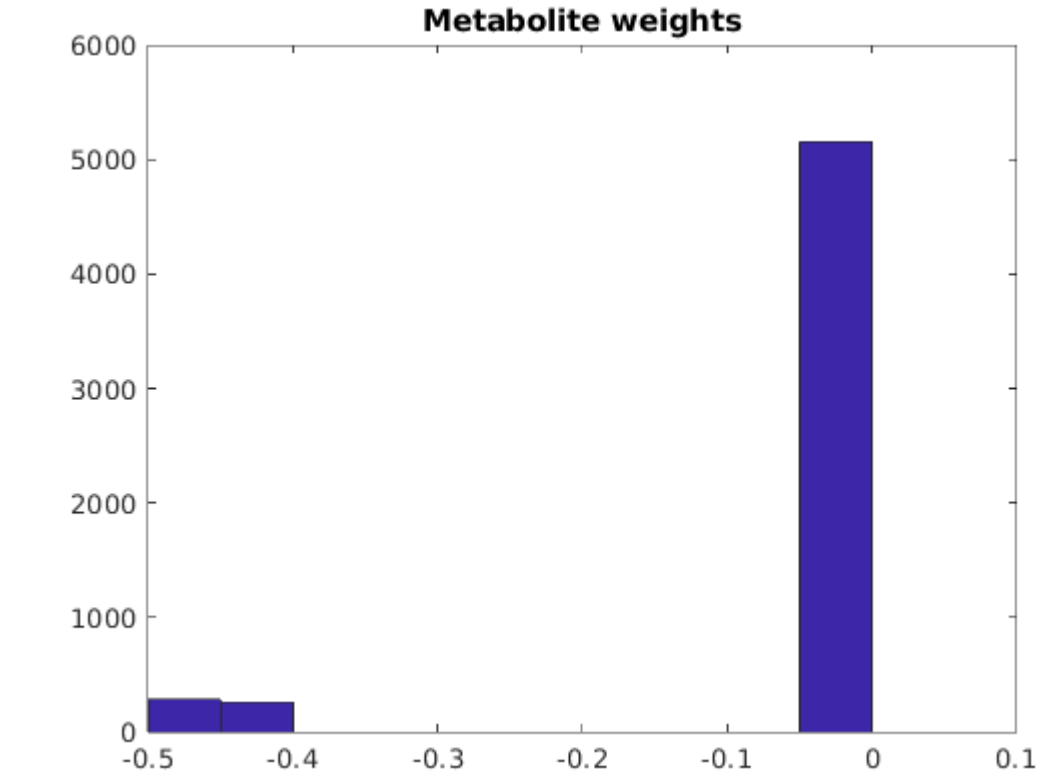

```
--- thermoKernel END ----
```

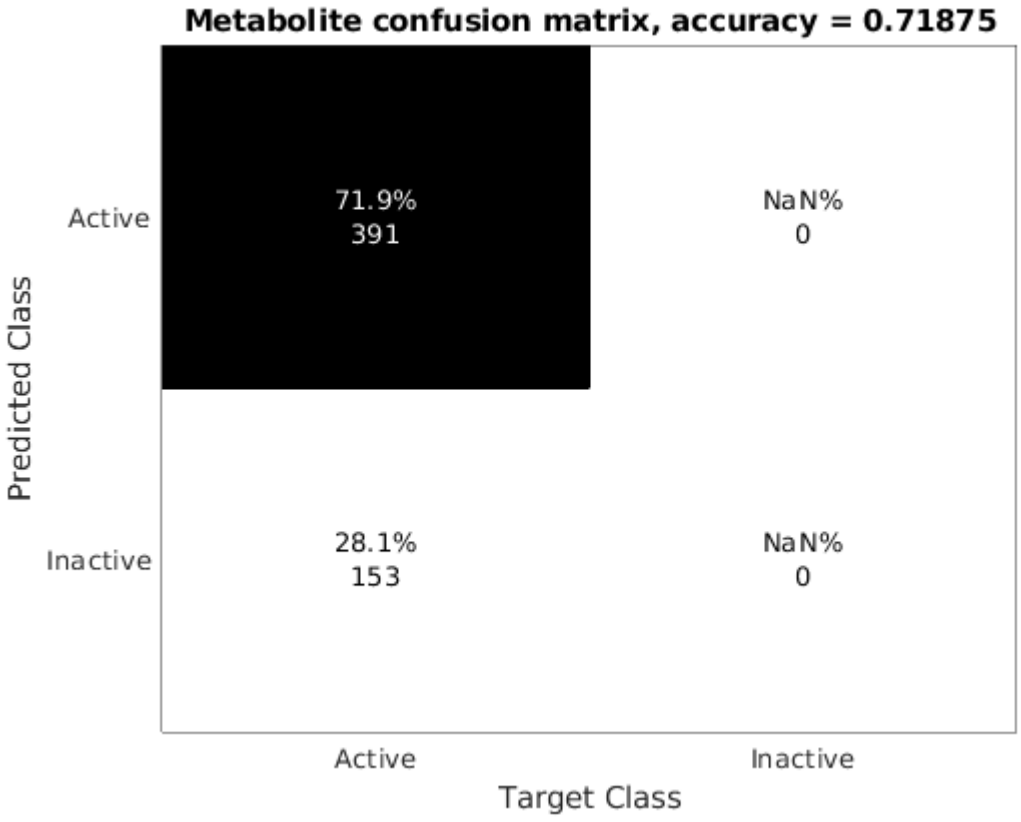
```
[nMet,nRxn]=size(tissueModel.S)
```

```
nMet = 1448
nRxn = 1753
```

Compare the target versus predicted model

```
plotThermoKernelExtractStats(stoichFluxThermoConsistModel,
activeInactiveRxn, rxnWeightsRed, presentAbsentMet, metWeightsRed,
thermoModelMetBool, thermoModelRxnBool)
```





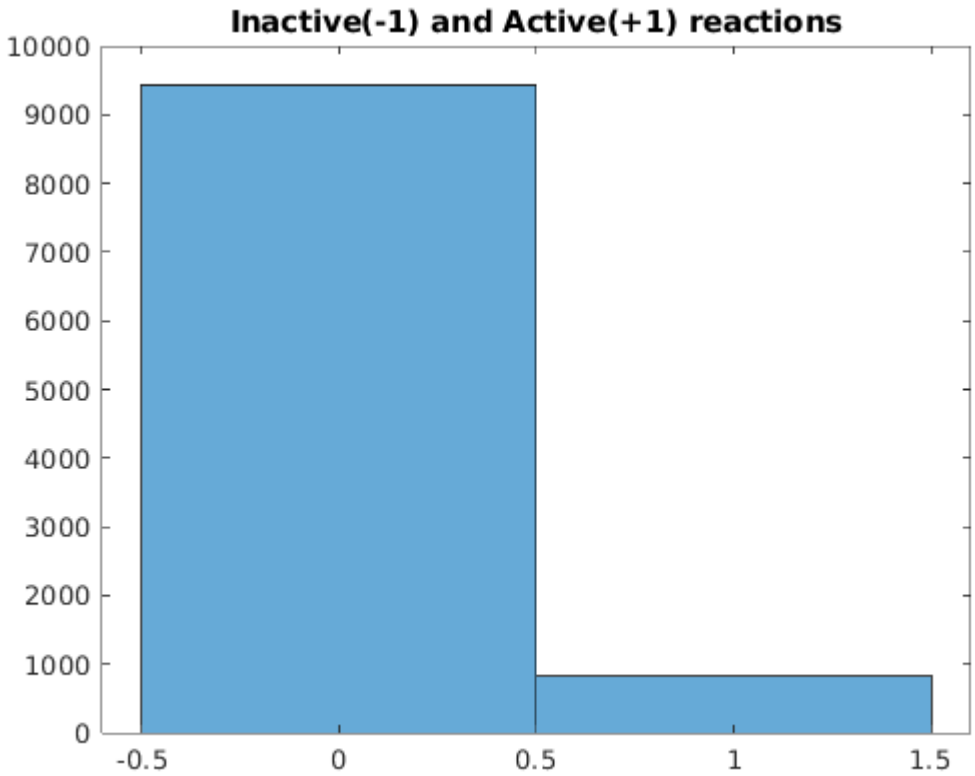
Submodel with just active reactions specified

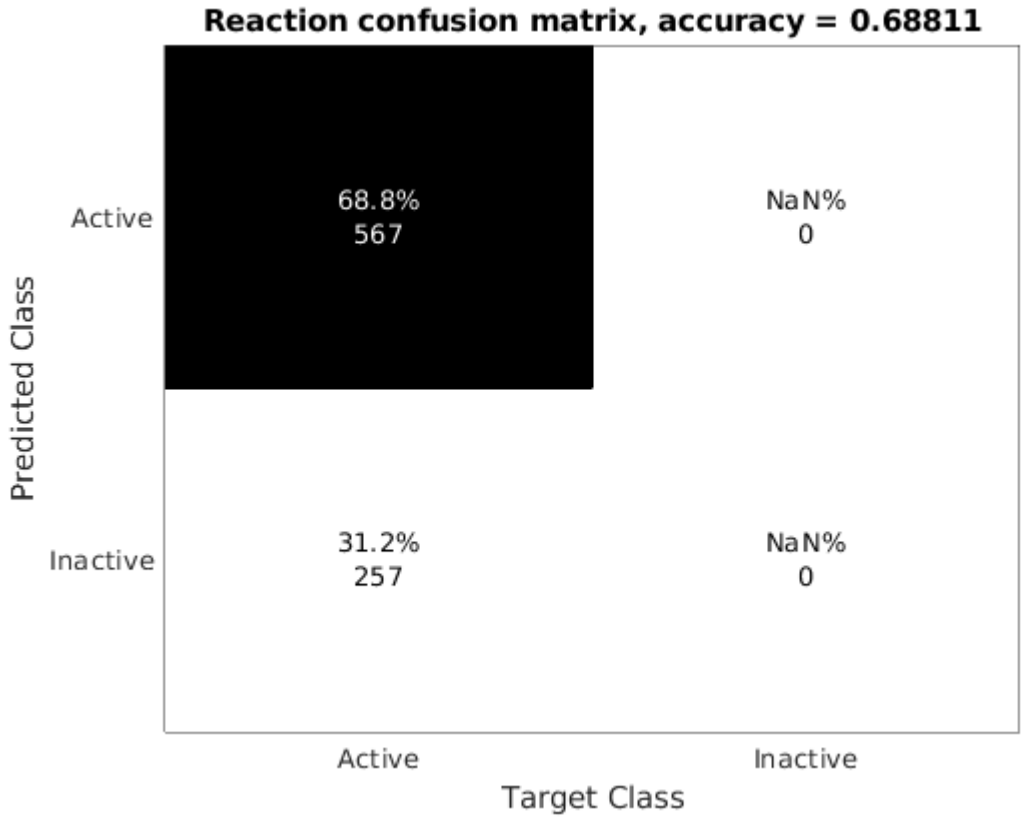
```
rxnWeightsRed=rxnWeightsTmp;
metWeightsRed=metWeightsTmp*0;
rxnWeightsRed(rxnWeightsRed>=0)=0;
[tissueModel, thermoModelMetBool, thermoModelRxnBool] =
thermoKernel(stoichFluxThermoConsistModel, activeInactiveRxn, rxnWeightsRed,
presentAbsentMet, metWeightsRed,param);
```

```
--- thermoKernel START ----
thermoKernel parameters:
                  printLevel: 1
                 relaxBounds: 0
           acceptRepairedFlux: 1
                           n: 200
    normalizeZeroNormWeights: 0
                 formulation: 'pqzwrs'
                     epsilon: 1.0000e-06
            removeOrphanGenes: 1
               nbMaxIteration: 30
                        nMax: 20
             iterationMethod: 'greedyRandom'

            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5000
                      theta: 0.5000
            regularizeOuter: 1
                    epsilon: 1.0000e-06
                printLevel: 1
               relaxBounds: 0
         acceptRepairedFlux: 1
    thermoConsistencyMethod: 'cycleFreeFlux'
                    bigNum: 10000
                     debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
        -0.5 = min(g0), the local weight on zero-norm of internal reaction rate.
           0 = max(g0), the local weight on zero-norm of internal reaction rate.
          -0 = min(h0), the local weight on zero-norm of metabolite production rate.
          -0 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

0 min cardinality variables:
         NaN mean(c(p))             NaN min(c(p))             NaN max(c(p))
           1 lambda0             NaN min(k)             NaN max(k)
           0 lambda1             NaN min(o(p))             NaN max(o(p))

824 max cardinality variables:
          -0 mean(c(q))              -0 min(c(q))              -0 max(c(q))
           1 delta0             0.4 min(d)             0.5 max(d)
           0 delta1               0 min(o(q))               0 max(o(q))

37723 cardinality free variables:
        0.45 mean(c(r))              -0 min(c(r))               1 max(c(r))
           0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.6384e+05    -8.4e+07         8.4         8.5           0           0           0    -3.118
   2    0.75      1.1692      -0.091         8.3         8.4           0           0           0    -3.548
   3    1.12     0.15183      -0.062         8.3         8.4           0           0           0    -3.506
   4    1.69    0.092443      -0.084         8.2         8.4           0           0           0    -3.506
```

```
   5    2.53     0.44167       -0.14         8.1         8.5           0           0           0    -3.506
   6    3.80     0.31473       -0.25         7.8         8.5           0           0           0    -3.956
   7    5.70      1.8987       -0.76           7         9.6           0           0           0    -4.903
   8    8.54      1.0204        -1.2         5.8         9.4           0           0           0    -5.394
   9   12.81      1.4536        -1.1         4.8          10           0           0           0    -5.832
  10   19.22     0.67982       -0.85         3.9         9.8           0           0           0    -5.832
  11   28.83     0.60377       -0.54         3.4         9.2           0           0           0    -5.832
  12   43.25     0.45629       -0.35           3         8.9           0           0           0    -5.832
  13   64.87     0.57354       -0.22         2.8         8.7           0           0           0    -5.832
  14   97.31      0.4286       -0.13         2.7         8.5           0           0           0    -5.832
  15  145.96       0.145      -0.036         2.7         8.5           0           0           0    -5.832
  16  218.95     0.11648      -0.019         2.6         8.5           0           0           0    -5.832
  17  328.42    0.083385      -0.012         2.6         8.5           0           0           0    -5.832
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
            warmStartMethod: 'random'
                formulation: 'pqzwrs'
            thetaMultiplier: 1.5000
                      theta: 0.5000
            regularizeOuter: 1
                    epsilon: 1.0000e-06
                 printLevel: 1
                relaxBounds: 0
          acceptRepairedFlux: 1
     thermoConsistencyMethod: 'cycleFreeFlux'
                      bigNum: 10000
                       debug: 0

optCardThermo objective data:
           1 = beta, the global weight on one-norm of internal reaction rate.
        -0.5 = min(g0), the local weight on zero-norm of internal reaction rate.
           0 = max(g0), the local weight on zero-norm of internal reaction rate.
          -0 = min(h0), the local weight on zero-norm of metabolite production rate.
          -0 = max(h0), the local weight on zero-norm of metabolite production rate.

optimizeCardinality objective data:

0 min cardinality variables:
         NaN mean(c(p))             NaN min(c(p))             NaN max(c(p))
           1 lambda0              NaN min(k)              NaN max(k)
           0 lambda1              NaN min(o(p))              NaN max(o(p))

824 max cardinality variables:
          -0 mean(c(q))              -0 min(c(q))              -0 max(c(q))
           1 delta0             0.4 min(d)             0.5 max(d)
           0 delta1               0 min(o(q))               0 max(o(q))

37723 cardinality free variables:
        0.45 mean(c(r))              -0 min(c(r))               1 max(c(r))
           0 alpha1               0 min(o(r))               0 max(o(r))

 itn     theta    ||dx||     del_obj         obj      linear      ||x||0        a(x)      ||x||1      ||y|
   1    0.50  9.6834e+05    -8.4e+07         8.4         8.5           0           0           0    -3.540
   2    0.75       1.251      -0.098         8.3         8.4           0           0           0    -3.970
   3    1.12    0.091425      -0.056         8.3         8.4           0           0           0    -3.506
   4    1.69    0.092443      -0.084         8.2         8.4           0           0           0    -3.506
   5    2.53     0.44167       -0.14         8.1         8.5           0           0           0    -3.506
   6    3.80     0.31473       -0.25         7.8         8.5           0           0           0    -3.956
   7    5.70      1.8987       -0.76           7         9.6           0           0           0    -4.903
   8    8.54      1.0204        -1.2         5.8         9.4           0           0           0    -5.394
   9   12.81      1.4536        -1.1         4.8          10           0           0           0    -5.832
  10   19.22     0.67982       -0.85         3.9         9.8           0           0           0    -5.832
  11   28.83     0.60377       -0.54         3.4         9.2           0           0           0    -5.832
```

```
  12   43.25     0.45629       -0.35          3         8.9          0          0          0    -5.832
  13   64.87     0.57354       -0.22        2.8         8.7          0          0          0    -5.832
  14   97.31      0.4286       -0.13        2.7         8.5          0          0          0    -5.832
  15  145.96       0.145      -0.036        2.7         8.5          0          0          0    -5.832
  16  218.95     0.11648      -0.019        2.6         8.5          0          0          0    -5.832
  17  328.42    0.083385      -0.012        2.6         8.5          0          0          0    -5.832
 itn     theta    ||dx||     del_obj         obj      linear      ||x||0       a(x)      ||x||1      ||y|
     Optimise cardinality reached the stopping criterion. Finished.
100.00% thermodynamically feasible internal fluxes (checked by cycleFreeFlux method).
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
    1       111              1.00              0.02       74              1.24               NaN      gre
    2       110              1.00              0.02       76              1.24               NaN      gre
    3       132              1.00              0.03       93              1.22               NaN      gre
    4       127              1.00              0.04       94              1.18               NaN      gre
    5       215              1.00              0.10      152              1.24               NaN      gre
    6       190              1.00              0.15      138              1.23               NaN      gre
    7       247              1.00              0.21      175              1.29               NaN      gre
    8       268              1.00              0.28      193              1.34               NaN      gre
    9       300              1.00              0.36      217              1.27               NaN      gre
   10       284              1.00              0.43      201              1.42               NaN      gre
   11       234              1.00              0.47      162              1.37               NaN      gre
   12       325              1.00              0.53      235              1.39               NaN      gre
   13       217              1.00              0.56      155              1.39               NaN      gre
   14       223              1.00              0.59      158              1.29               NaN      gre
   15       197              1.00              0.60      137              1.37               NaN      gre
   16       136              1.00              0.61       94              1.29               NaN      gre
   17       189              1.00              0.63      135              1.39               NaN      gre
   18       222              1.00              0.65      148              1.47               NaN      gre
   19       316              1.00              0.68      219              1.42               NaN      gre
   20       155              1.00              0.69      114              1.34               NaN      gre
iter.  nz.flux.%it.feas.int.flux.   %feas.inc.flux.  nz.prod. %it.feas.nz.prod.   %feas.inc.prod.       fo
thermoKernel terminating early: n = nMax = 20
```





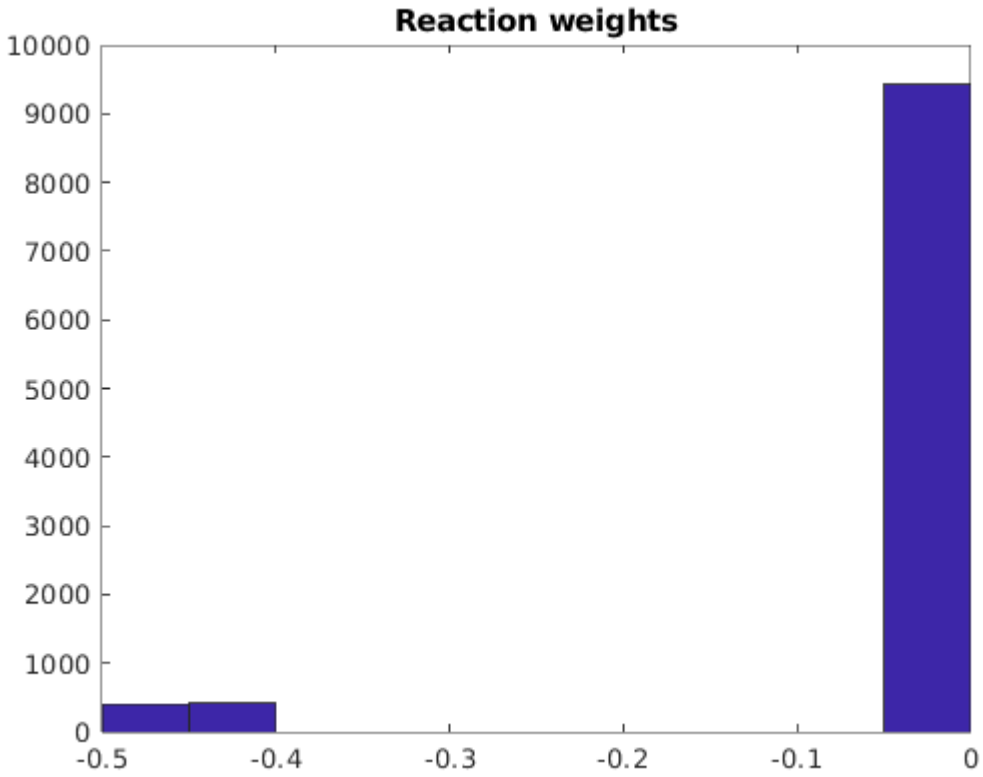

```
--- thermoKernel END ----
```

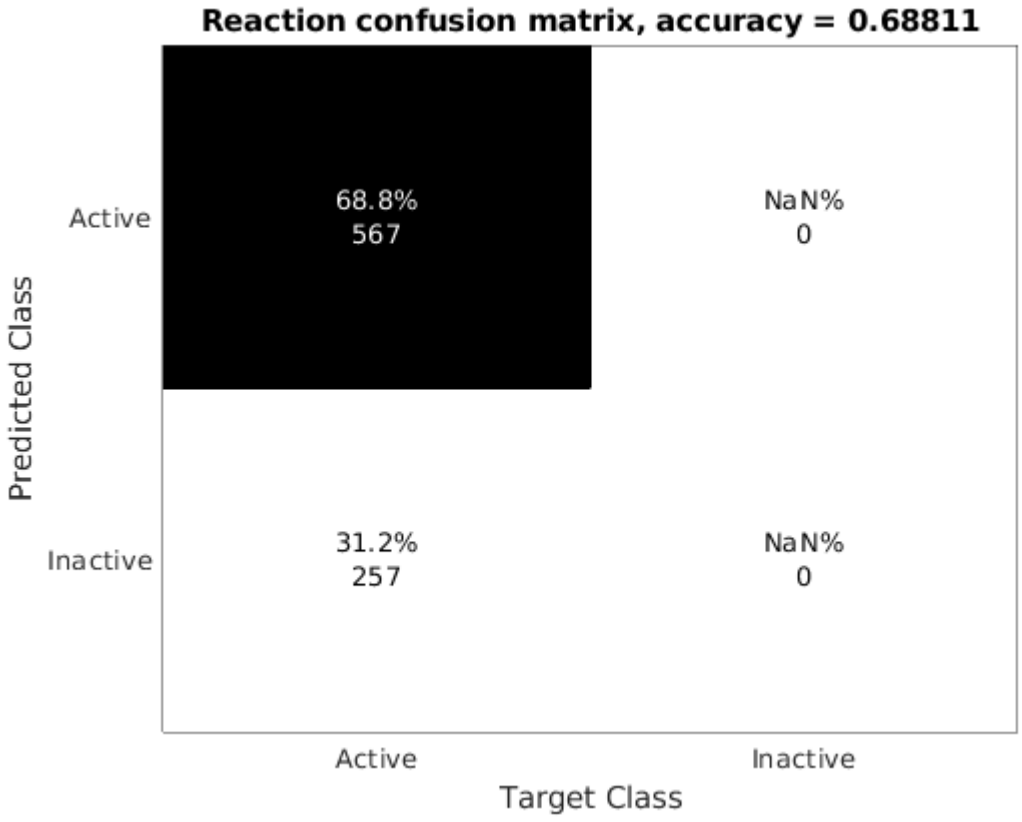
```
[nMet,nRxn]=size(tissueModel.S)
```

```
nMet = 1449
nRxn = 1915
```

Compare the target versus predicted model

```
plotThermoKernelExtractStats(stoichFluxThermoConsistModel,
activeInactiveRxn, rxnWeightsRed, presentAbsentMet, metWeightsRed,
thermoModelMetBool, thermoModelRxnBool)
```





Acknowledgments

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REFERENCES

[1] Ronan M T Fleming, Hulda S Haraldsdottir, Le Hoai Minh, Phan Tu Vuong, Thomas Hankemeier, Ines Thiele, Cardinality optimization in constraint-based modelling: application to human metabolism, *Bioinformatics*, Volume 39, Issue 9, September 2023, btad450, https://doi.org/10.1093/bioinformatics/btad450

[2] Preciat, G., Wegrzyn, A. B., Luo, X., Thiele, I., Hankemeier, T., & Fleming, R. M. T. (2025). XomicsToModel: omics data integration and generation of thermodynamically consistent metabolic models. Nature Protocols. https://doi.org/10.1038/s41596-025-01288-9