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```
In [37]: import numpy as np
import pandas as pd
import matplotlib.pyplot as plt
import matplotlib
import subprocess
import re
import os
from IPython.core.display import display
pd.set_option('display.max_columns', None, 'display.max_rows', None)
#from ipynb_latex_setup import *
%matplotlib inline
#%matplotlib pdf
#matplotlib.matplotlib_fname()
#import matplotlib.rcsetup as rcsetup
#print(rcsetup.all_backends)
T = 298.15
R = 8.314e-03
RT = R*T
N_avogadro = 6.022140857e+23
VolCell = 1.0e-15
Concentration2Count = N_avogadro * VolCell
concentration_increment = 1/(N_avogadro*VolCell)
```

Change directories to where the simulation data is:

```
In [38]: # Comment/uncomment to change to your directory, if needed:
cwd = os.getcwd()
mydir = cwd+'/simulation_data'
if 'simulation_data' not in mydir:
    os.chdir(mydir)
```

Boltzmann Simulation

A beta version of the simulation code is available on GitHub at <https://github.com/PNNL-CompBio/Boltzmann> (<https://github.com/PNNL-CompBio/Boltzmann>).

Pentose phosphate + Glycolysis + TCA cycle

Model input file (Reactions):

```
In [39]: cat neurospora_pentose_phos.glycolysis.tca.2.dat

REACTION ME1m
LEFT      (S)-MALATE + NAD+
RIGHT     pyruvate + NADH + CO2
LEFT_COMPARTMENT MITOCHONDRIA
RIGHT_COMPARTMENT MITOCHONDRIA
ENZYME_LEVEL      0.0
//
REACTION ME2m
LEFT      (S)-MALATE + NADP+
RIGHT     PYRUVATE + NADPH + CO2
LEFT_COMPARTMENT MITOCHONDRIA
RIGHT_COMPARTMENT MITOCHONDRIA
ENZYME_LEVEL      0.0
//
REACTION CSm
LEFT      OXALOACETATE + ACETYL-COA + H2O
RIGHT     CITRATE + COA
LEFT_COMPARTMENT MITOCHONDRIA
RIGHT_COMPARTMENT MITOCHONDRIA
COMMENT PH = 7.0, IONIC STRENGTH = 0.15 M
//
REACTION ACONTm
LEFT      CITRATE
RIGHT     ISOCITRATE
LEFT_COMPARTMENT MITOCHONDRIA
RIGHT_COMPARTMENT MITOCHONDRIA
COMMENT PH = 7.0, IONIC STRENGTH = 0.15 M
COMMENT DON't split into two reactions if max entropy is being used
- only one enzyme needs to be epressed
//
REACTION ICDHxm
LEFT      ISOCITRATE + NAD+
```

```

RIGHT    2-OXOGLUTARATE + NADH + CO2
LEFT_COMPARTMENT MITOCHONDRIA
RIGHT_COMPARTMENT MITOCHONDRIA
COMMENT PH = 7.0, IONIC STRENGTH = 0.15 M
//
REACTION AKGDm
LEFT      2-OXOGLUTARATE + COA + NAD+
RIGHT     SUCCINYL-COA + CO2 + NADH
LEFT_COMPARTMENT MITOCHONDRIA
RIGHT_COMPARTMENT MITOCHONDRIA
COMMENT PH = 7.0, IONIC STRENGTH = 0.15 M
//
REACTION SUCOASm
LEFT      SUCCINYL-COA + ADP + Orthophosphate
RIGHT     SUCCINATE + ATP + COA
LEFT_COMPARTMENT MITOCHONDRIA
RIGHT_COMPARTMENT MITOCHONDRIA
COMMENT PH = 7.0, IONIC STRENGTH = 0.15 M
//
REACTION SUCD1m
LEFT      SUCCINATE + redox1
RIGHT     FUMARATE + redox2
LEFT_COMPARTMENT MITOCHONDRIA
RIGHT_COMPARTMENT MITOCHONDRIA
COMMENT PH = 7.0, IONIC STRENGTH = 0.15 M
//
REACTION FUMm
LEFT      FUMARATE + H2O
RIGHT     (S)-MALATE
LEFT_COMPARTMENT MITOCHONDRIA
RIGHT_COMPARTMENT MITOCHONDRIA
COMMENT PH = 7.0, IONIC STRENGTH = 0.15 M
//
REACTION MDHm
LEFT      (S)-MALATE + NAD+
RIGHT     OXALOACETATE + NADH
LEFT_COMPARTMENT MITOCHONDRIA
RIGHT_COMPARTMENT MITOCHONDRIA
COMMENT PH = 7.0, IONIC STRENGTH = 0.15 M
//
REACTION GAPD
LEFT      D-GLYCERALDEHYDE-3-PHOSPHATE + ORTHOPHOSPHATE + NAD+
RIGHT     3-Phospho-D-glyceroyl_phosphate + NADH
ENZYME_LEVEL    1.0
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
PATHWAY GLUCONEOGENESIS, GLYCOLYSIS
COMMENTS  From Dennis
//
REACTION PGK
LEFT      3-Phospho-D-glyceroyl_phosphate + ADP
RIGHT     3-PHOSPHO-D-GLYCERATE + ATP
LEFT_COMPARTMENT CYTOSOL

```

```

RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      1.0
PATHWAY GLUCONEOGENESIS, GLYCOLYSIS, CALVIN CYCLE
COMMENTS From Dennis
//
REACTION TPI
LEFT      Glycerone_phosphate
RIGHT     D-GLYCERALDEHYDE-3-PHOSPHATE
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      1.0
COMMENT From Equilibrator
PATHWAY CALVIN CYCLE, GLYCOLYSIS
//
REACTION MDH
LEFT      (S)-MALATE + NAD+
RIGHT     OXALOACETATE + NADH
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
COMMENT PH = 7.0, IONIC STRENGTH = 0.15 M
COMMENT Cytosolic
//
REACTION PEP_Carboxylase
LEFT      oxaloacetate + orthophosphate
RIGHT     phosphoenolpyruvate + CO2 + H2O
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY GLUCONEOGENESIS
COMMENTS
//
REACTION PPCK
LEFT      oxaloacetate + ATP
RIGHT     phosphoenolpyruvate + ADP + CO2
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY GLUCONEOGENESIS
COMMENTS
//
REACTION FBA
LEFT      D-FRUCTOSE_1,6-BISPHOSPHATE
RIGHT     Glycerone_phosphate + D-GLYCERALDEHYDE-3-PHOSPHATE
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      1.0
PATHWAY GLUCONEOGENESIS, GLYCOLYSIS, CALVIN CYCLE
COMMENTS From Dennis
//
REACTION FBP
LEFT      D-FRUCTOSE_6-PHOSPHATE + Orthophosphate
RIGHT     H2O + D-FRUCTOSE_1,6-BISPHOSPHATE

```

```

LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY GLUCONEOGENESIS, CALVIN CYCLE
COMMENTS  From Dennis
//
REACTION TKT2
LEFT      D-FRUCTOSE_6-PHOSPHATE + D-GLYCERALDEHYDE-3-PHOSPHATE
RIGHT     D-ERYTHROSE-4-PHOSPHATE + D-XYLULOSE-5-PHOSPHATE
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY CALVIN CYCLE, PENTOSE PHOSPHATE PATHWAY, RUBISCO SHUNT
COMMENT  From Equilibrator pH 7.5, IS 0.15
//
REACTION RPE
LEFT      D-XYLULOSE-5-PHOSPHATE
RIGHT     D-RIBULOSE-5-PHOSPHATE
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY CALVIN CYCLE, PENTOSE PHOSPHATE PATHWAY, RUBISCO SHUNT
COMMENT  From Dennis
//
REACTION Xylulokinase
LEFT      D-XYLULOSE + ATP
RIGHT     D-XYLULOSE-5-PHOSPHATE + ADP
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY CALVIN CYCLE, PENTOSE PHOSPHATE PATHWAY, RUBISCO SHUNT
COMMENT
//
REACTION PYK_org
LEFT      ADP + PHOSPHOENOLPYRUVATE
RIGHT     PYRUVATE + ATP
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY GLYCOLYSIS, PYRUVATE METABOLISM, RUBISCO SHUNT
COMMENT  From Dennis
//
REACTION PYK
LEFT      ADP + PHOSPHOENOLPYRUVATE
RIGHT     PYRUVATE + ATP
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      1.0
PATHWAY GLYCOLYSIS, PYRUVATE METABOLISM, RUBISCO SHUNT
COMMENT  From Dennis
//
REACTION RPI
LEFT      D-RIBOSE-5-PHOSPHATE

```

```

RIGHT    D-RIBULOSE-5-PHOSPHATE
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY CALVIN CYCLE, PENTOSE PHOSPHATE PATHWAY, RUBISCO SHUNT
COMMENT  From Dennis
//
REACTION TKT1
LEFT      SEDOHEPTULOSE_7-PHOSPHATE + D-GLYCERALDEHYDE-3-PHOSPHATE
RIGHT     D-RIBOSE-5-PHOSPHATE + D-XYLULOSE-5-PHOSPHATE
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY CALVIN CYCLE, PENTOSE PHOSPHATE PATHWAY, RUBISCO SHUNT
COMMENT  From Equilibrator pH 7.5, IS 0.15
//
REACTION TALA
LEFT      D-GLYCERALDEHYDE-3-PHOSPHATE + SEDOHEPTULOSE_7-PHOSPHATE
RIGHT     D-FRUCTOSE_6-PHOSPHATE + D-ERYTHROSE-4-PHOSPHATE
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY RUBISCO SHUNT, PENTOSE PHOSPHATE PATHWAY
COMMENT  From Equilibrator pH 7.5 IS 0.15
//
REACTION PGM
LEFT      3-PHOSPHO-D-GLYCERATE
RIGHT     2-PHOSPHO-D-GLYCERATE
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      1.0
PATHWAY GLUCONEOGENESIS, RUBISCO SHUNT, GLYCOLYSIS I
COMMENTS  From Dennis
//
REACTION ENO
LEFT      2-PHOSPHO-D-GLYCERATE
RIGHT     PHOSPHOENOLPYRUVATE + H2O
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      1.0
PATHWAY GLUCONEOGENESIS, RUBISCO SHUNT, GLYCOLYSIS I
COMMENTS  From Dennis
//
# Pentose Phosphate reactions (oxidative branch):
REACTION GND
LEFT      NADPH + D-RIBULOSE-5-PHOSPHATE + CO2
RIGHT     NADP+ + 6-PHOSPHO-D-GLUCONATE
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
PATHWAY PENTOSE PHOSPHATE PATHWAY
ENZYME_LEVEL      0.0
//
REACTION PGL

```

```

LEFT  6-PHOSPHO-D-GLUCONATE
RIGHT D-Glucono-1,5-lactone_6-phosphate + H2O
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
COMMENT From Dennis
PATHWAY PENTOSE PHOSPHATE PATHWAY
ENZYME_LEVEL      0.0
//
REACTION HEX1
LEFT      BETA-D-GLUCOSE + ATP
RIGHT     BETA-D-GLUCOSE-6-PHOSPHATE + ADP
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
NREGULATION ATP 2.80e-03 10
ENZYME_LEVEL      1.0
PATHWAY GLUCONEOGENESIS, (GLYCOLYSIS-YEAST), GLYCOLYSIS
COMMENTS  From Dennis
//
REACTION PGI
LEFT      BETA-D-GLUCOSE-6-PHOSPHATE
RIGHT     D-FRUCTOSE_6-PHOSPHATE
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      1.0
PATHWAY GLUCONEOGENESIS, (GLYCOLYSIS-YEAST), GLYCOLYSIS
COMMENTS  From Dennis
//
# Gluconeogenesis reactions :
REACTION G6PDH2r
LEFT      BETA-D-GLUCOSE-6-PHOSPHATE + NADP+
RIGHT     D-Glucono-1,5-lactone_6-phosphate + NADPH
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
COMMENT  From Dennis
PATHWAY GLUCONEOGENESIS
//
# Glycolysis reactions :
REACTION PFK
LEFT      D-FRUCTOSE_6-PHOSPHATE + ATP
RIGHT     ADP + D-FRUCTOSE_1,6-BISPHOSPHATE
LEFT_COMPARTMENT CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
NREGULATION ATP 2.80e-03 10
ENZYME_LEVEL      1.0
PATHWAY GLYCOLYSIS
COMMNENT From Equilibrator
//
REACTION PYRt2m
LEFT      PYRUVATE
RIGHT     PYRUVATE
DGZERO    -5.94
COMMENT   -5.94

```

```

DGZERO-UNITS      KJ/MOL
LEFT_COMPARTMENT  CYTOSOL
RIGHT_COMPARTMENT MITOCHONDRIA
ENZYME_LEVEL      1.0
COMMENT PYRUVATE TRANSPORT INTO MITOCHONDRIA - Assuming free energy
difference = \Delta -RT \Delta pH = -RT log 10
//
# Acetyl CoA synthesis
REACTION PDHm
LEFT      COA + NAD+ + PYRUVATE
RIGHT     ACETYL-COA + CO2 + NADH
LEFT_COMPARTMENT  MITOCHONDRIA
RIGHT_COMPARTMENT MITOCHONDRIA
ENZYME_LEVEL      1.0
NREGULATION ACETYL-COA 1.00e-03 20
PATHWAY ACETYL COA BIOSYNTHESIS
COMMENT PYRUVATE DEHYDROGENASE SUMMARY REACTION
//
REACTION ICL
LEFT isocitrate
RIGHT  glyoxylate + succinate
LEFT_COMPARTMENT  GLYOXYSOME
RIGHT_COMPARTMENT GLYOXYSOME
ENZYME_LEVEL      0.0
PATHWAY glyoxylate shunt
COMMENT
//
REACTION MAS
LEFT acetyl-CoA + glyoxylate + H2O
RIGHT  (S)-MALATE + COA
LEFT_COMPARTMENT  GLYOXYSOME
RIGHT_COMPARTMENT GLYOXYSOME
ENZYME_LEVEL      0.0
PATHWAY glyoxylate shunt
COMMENT
//
REACTION PYRDC
LEFT pyruvate
RIGHT acetaldehyde + CO2
LEFT_COMPARTMENT  CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY fermentation
COMMENT
//
REACTION ALDD2y
LEFT acetaldehyde + NADP+ + H2O
RIGHT acetate + NADPH
LEFT_COMPARTMENT  CYTOSOL
RIGHT_COMPARTMENT CYTOSOL
ENZYME_LEVEL      0.0
PATHWAY fermentation
COMMENT

```



```
//  
REACTION ALCD2X_copy1  
LEFT acetaldehyde + NADH  
RIGHT ethanol + NAD+  
LEFT_COMPARTMENT CYTOSOL  
RIGHT_COMPARTMENT CYTOSOL  
ENZYME_LEVEL 0.0  
PATHWAY fermentation  
COMMENT  
//  
REACTION LactateDehydrogenase  
LEFT pyruvate + NADH  
RIGHT lactate + NAD+  
LEFT_COMPARTMENT CYTOSOL  
RIGHT_COMPARTMENT CYTOSOL  
ENZYME_LEVEL 0.0  
PATHWAY fermentation  
COMMENT  
//
```

Boltzmann Input File:

In [40]: `cat neurospora_pentose_phos.glycolysis.tca_reg.in`

```
RXN_FILE neurospora_pentose_phos.glycolysis.tca.2.dat
INIT_FILE neurospora_pentose_phos.glycolysis.tca.2_reg.rstrt
INIT_FILE yeast_centralMetab_concs2.in
INIT_FILE neurospora_pentose_phos.glycolysis.tca_concs.in
INIT_FILE neurospora_pentose_phos.glycolysis.tca.2_reg.rstrt
LOG_FILE neurospora_pentose_phos.glycolysis.tca.2_reg.log
OUT_FILE neurospora_pentose_phos.glycolysis.tca.2_reg.out
USE_DEQ 1
NO_ROUND_FROM_DEQ 1
ODE_T_FINAL 100000
DELTA_CONCS_CHOICE 13
DELTA_CONCS_CHOICE 8
DERIV_THRESH 5.0e-17
ODE_RXN_VIEW_FREQ 1
WARMUP_STEPS 00000000
RECORD_STEPS 000000
TEMP_KELVIN 298.15
PH 7.0
IONIC_STRENGTH 0.15
PRINT_OUTPUT 2
CONCS_OR_COUNTS 3
RXN_VIEW_FREQ 100
COUNT_VIEW_FREQ 100
LKLHD_VIEW_FREQ 100
USE_BULK_WATER 1
USE_REGULATION 1
USE_ENZYME_LEVELS 1
USE_PSEUDOISOMERS 1
USE_DGZERO 1
USE_ACTIVITIES 1
NUM_METABOLIC_GROUPS 1
```

Models and Simulations

Model Without Regulation

Run Deterministic Simulation

Uncomment the lines below to run Boltzmann from the Jupyter notebook. We prefer not to run from the notebook because it may take several runs before the system converges.

```
In [41]: #args = ("boltzmann", "neurospora_pentose_phos.glycolysis.tca_no_reg.i
n")
#popen = subprocess.Popen(args, stdout=subprocess.PIPE)
#popen.wait()
#output = popen.stdout.read()
#print(output)
```

Derivatives from ODE thermodynamic optimization simulation

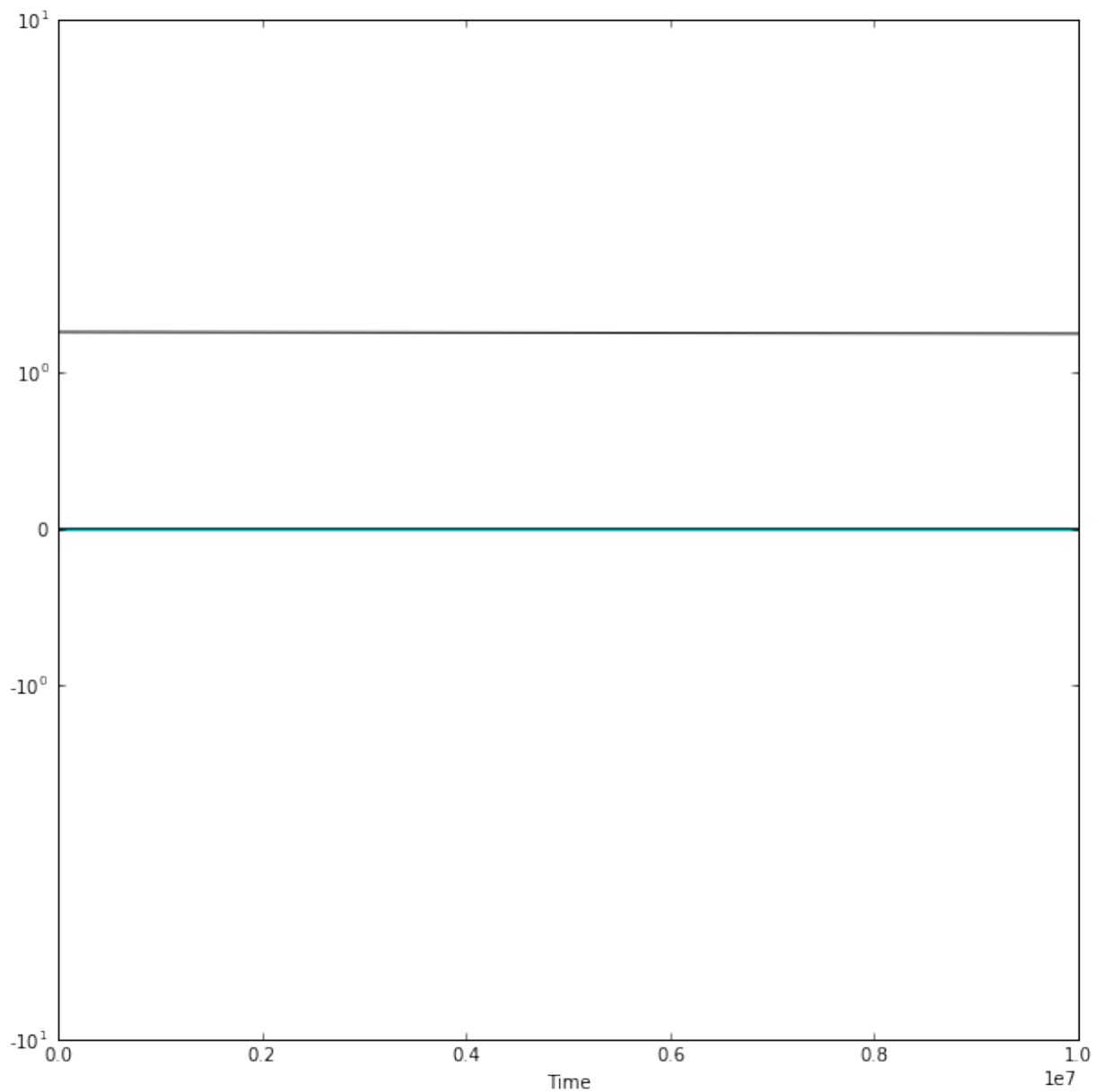
For the maximum entropy optimization without any regulation, multiple rounds of optimization were used to obtain sufficiently converged derivatives. The maximum entropy distribution has a wide range of metabolite concentrations, from 10^{-22} for oxaloacetate to $10^6 M$ for fructose-1,6-bisphosphate and $10^9 M$ for acetyl-CoA. Despite these large differences, derivatives remain stable until the the derivatives for acetyl-CoA go below 0.05. At this point the solver becomes unstable because even a small time step results in a large change the derivative. However, the derivatives are sufficiently converged at this point that the loss function can be applied to the metabolite levels to infer reactions that should be regulated. To put this another way, it does not matter for the purpose of inferring regulation if the acetyl-CoA concentraion is $1.0 \cdot 10^9$ or $6.0 \cdot 10^9$, both are well beyond the expected millimolar level.

```
In [42]: # Read boltzmann stochastic reaction likelihoods
ode_derivatives = pd.read_table('neurospora_pentose_phos.glycolysis.tca.2_no_reg.ode_dconcs', header=0, index_col=0)
# Get the absolute value of the derivatives:
abs_derivatives = np.abs(ode_derivatives.iloc[-1,:])
display(abs_derivatives.sort_values())
#plt.figure();ode_derivatives.iloc[-5000:-1500,:].plot(legend=False,logx=True,logy=False,figsize=(10, 10))
plt.figure();ode_derivatives.plot(legend=False,logx=False,logy=False,figsize=(10, 10))
plt.yscale('symlog')
#plt.legend(bbox_to_anchor=(1.35, 1.05),bbox_transform=plt.gcf().transFigure)
```

(S)-MALATE:CYTOSOL	0.000000e+00
NADP+:CYTOSOL	0.000000e+00
NADPH:CYTOSOL	0.000000e+00
ORTHOPHOSPHATE:CYTOSOL	0.000000e+00
SEDOHEPTULOSE_7-PHOSPHATE:CYTOSOL	0.000000e+00
(S)-MALATE:GLYOXYSOME	0.000000e+00
ACETYL-COA:GLYOXYSOME	0.000000e+00
COA:GLYOXYSOME	0.000000e+00
GLYOXYLATE:GLYOXYSOME	0.000000e+00
ISOCITRATE:GLYOXYSOME	0.000000e+00
SUCCINATE:GLYOXYSOME	0.000000e+00
ADP:MITOCHONDRIA	0.000000e+00
ATP:MITOCHONDRIA	0.000000e+00
CO2:MITOCHONDRIA	0.000000e+00
COA:MITOCHONDRIA	0.000000e+00
NAD+:MITOCHONDRIA	0.000000e+00

NADH:MITOCHONDRIA	0.000000e+00
NADP+:MITOCHONDRIA	0.000000e+00
NADPH:MITOCHONDRIA	0.000000e+00
ORTHOPHOSPHATE:MITOCHONDRIA	0.000000e+00
REDOX1:MITOCHONDRIA	0.000000e+00
REDOX2:MITOCHONDRIA	0.000000e+00
NADH:CYTOSOL	0.000000e+00
NAD+:CYTOSOL	0.000000e+00
OXALOACETATE:CYTOSOL	0.000000e+00
CO2:CYTOSOL	0.000000e+00
LACTATE:CYTOSOL	0.000000e+00
D-ERYTHROSE-4-PHOSPHATE:CYTOSOL	0.000000e+00
ADP:CYTOSOL	0.000000e+00
D-GLUCONO-1,5-LACTONE_6-PHOSPHATE:CYTOSOL	0.000000e+00
ATP:CYTOSOL	0.000000e+00
D-RIBOSE-5-PHOSPHATE:CYTOSOL	0.000000e+00
ACETATE:CYTOSOL	0.000000e+00
ACETALDEHYDE:CYTOSOL	0.000000e+00
6-PHOSPHO-D-GLUCONATE:CYTOSOL	0.000000e+00
D-XYLULOSE:CYTOSOL	0.000000e+00
D-XYLULOSE-5-PHOSPHATE:CYTOSOL	0.000000e+00
ETHANOL:CYTOSOL	0.000000e+00
D-RIBULOSE-5-PHOSPHATE:CYTOSOL	0.000000e+00
BETA-D-GLUCOSE:CYTOSOL	0.000000e+00
ISOCITRATE:MITOCHONDRIA	8.881784e-16
(S)-MALATE:MITOCHONDRIA	8.881784e-16
2-OXOGLUTARATE:MITOCHONDRIA	8.881784e-16
FUMARATE:MITOCHONDRIA	8.881784e-16
OXALOACETATE:MITOCHONDRIA	2.664535e-15
SUCCINYL-COA:MITOCHONDRIA	2.664535e-15
SUCCINATE:MITOCHONDRIA	2.664535e-15
CITRATE:MITOCHONDRIA	2.664535e-15
D-GLYCERALDEHYDE-3-PHOSPHATE:CYTOSOL	9.769963e-15
GLYCERONE_PHOSPHATE:CYTOSOL	5.000445e-13
PHOSPHOENOLPYRUVATE:CYTOSOL	5.385301e-10
2-PHOSPHO-D-GLYCERATE:CYTOSOL	9.066543e-10
BETA-D-GLUCOSE-6-PHOSPHATE:CYTOSOL	9.005226e-09
D-FRUCTOSE_6-PHOSPHATE:CYTOSOL	1.085331e-08
3-PHOSPHO-D-GLYCERATE:CYTOSOL	6.297109e-08
3-PHOSPHO-D-GLYCEROYL_PHOSPHATE:CYTOSOL	2.462820e-07
PYRUVATE:CYTOSOL	5.712797e-07
PYRUVATE:MITOCHONDRIA	8.997118e-07
D-FRUCTOSE_1,6-BISPHOSPHATE:CYTOSOL	5.837993e-04
ACETYL-COA:MITOCHONDRIA	1.248513e+00
Name: 9999981.0, dtype: float64	

<matplotlib.figure.Figure at 0x11cc30470>



The values reported in the paper are those obtained just before the solver became unstable, at relative time 9.250000e+09.

```
In [43]: #display(ode_derivatives.loc[9.250000e+09].sort_values())
```

Thermodynamic Odds of Reaction from Deterministic Simulation

The thermodynamic likelihoods are read in from the output files, where the likelihood is $e^{A_\alpha/RT} = K_\alpha Q_\alpha^{-1}$. A_α is the reaction affinity for reaction α , K_α is the equilibrium constant, and Q_α is the reaction quotient.

```
In [44]: # Read boltzmann ODE reaction likelihoods
ode_likelihoods_timeseries = pd.read_table('neurospora_pentose_phos.glycolysis.tca.2_no_reg.ode_lklhd',header=1, index_col = 0, quoting=2)

temp = [x for x in ode_likelihoods_timeseries.columns if 'f_' in x]
ode_fwd_likelihoods_timeseries = ode_likelihoods_timeseries[temp]
temp = [x for x in ode_likelihoods_timeseries.columns if 'r_' in x]
ode_rev_likelihoods_timeseries = ode_likelihoods_timeseries[temp]

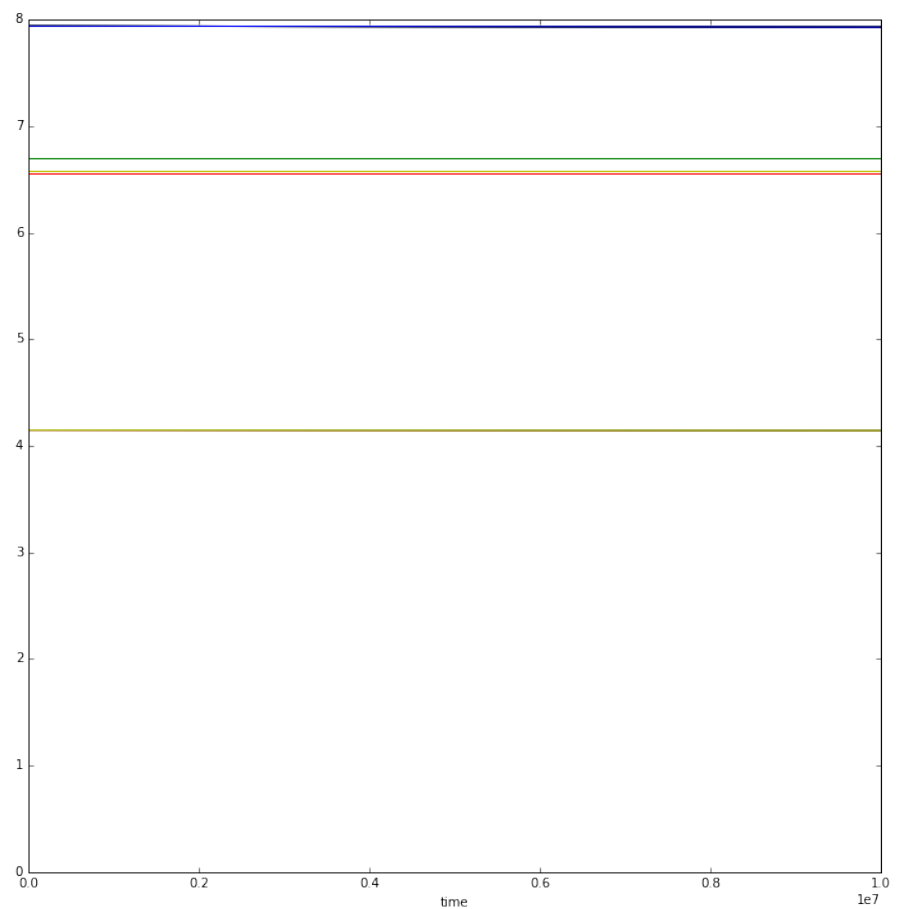
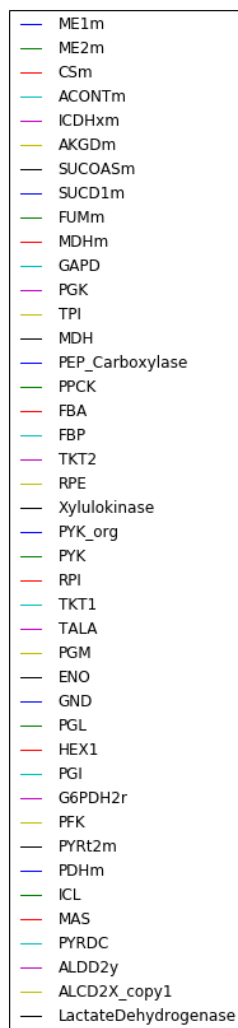
ode_fwd_likelihoods_timeseries.columns = [x.split("f_")[-1] for x in ode_fwd_likelihoods_timeseries.columns]
ode_rev_likelihoods_timeseries.columns = [x.split("r_")[-1] for x in ode_rev_likelihoods_timeseries.columns]

plt.figure()
ode_fwd_likelihoods_timeseries.plot(legend=False,logx=False,figsize=(12, 12))
#plt.yscale('log')
#plt.yscale('symlog')
plt.legend(bbox_to_anchor=(0.02, 0.9),bbox_transform=plt.gcf().transFigure)
#display(ode_fwd_likelihoods_timeseries.loc[:9.250000e+09].mean())
display(ode_fwd_likelihoods_timeseries.mean())
```

ME1m	0.000000
ME2m	0.000000
CSm	6.554156
ACONTm	6.703329
ICDHxm	6.582371
AKGDm	6.582372
SUCOASm	6.703335
SUCD1m	6.703332
FUMm	6.703327
MDHm	6.554156
GAPD	7.933921
PGK	7.933929
TPI	4.145176
MDH	0.000000
PEP_Carboxylase	0.000000
PPCK	0.000000
FBA	4.145176
FBP	0.000000
TKT2	0.000000
RPE	0.000000
Xylulokinase	0.000000
PYK_org	0.000000
PYK	7.933929
RPI	0.000000
TKT1	0.000000
TALA	0.000000
PGM	7.933930
ENO	7.933930
GND	0.000000
PGL	0.000000
HEX1	4.145744
PGI	4.145744
G6PDH2r	0.000000
PFK	4.145744
PYRt2m	7.933929
PDHm	7.933923
ICL	0.000000
MAS	0.000000
PYRDC	0.000000
ALDD2y	0.000000
ALCD2X_copy1	0.000000
LactateDehydrogenase	0.000000

dtype: float64

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As mentioned above, when the the derivatives for acetyl-CoA go below 0.05, the solver becomes unstable because even a small time step results in a large change the derivative. However, the likelihoods are sufficiently converged at this point that good estimates of the steady state reaction fluxes can be obtained.

Calculate Net Odds

The net odds of a reaction are proportional to the reaction flux. To get the steady state values of the likelihoods, we'll use the average of the last 30 steps before the solver became unstable (relative time from $9.247000\text{e}+09$ to $9.250000\text{e}+09$).


```
In [45]: #ode_likelihoods_steadystate = pd.DataFrame(data = ode_fwd_likelihoods_
_
timeseries.loc[9.247000e+09:9.250000e+09].mean(),
ode_likelihoods_steadystate = pd.DataFrame(data = ode_fwd_likelihoods_
timeseries.mean(),
index = ode_fwd_likelihoods_
_timeseries.columns,
columns =['Forward'])
#ode_likelihoods_steadystate['Reverse'] = ode_rev_likelihoods_timeseri
es.loc[9.247000e+09:9.250000e+09].mean()
ode_likelihoods_steadystate['Reverse'] = ode_rev_likelihoods_timeserie
s.mean()
#ode_likelihoods_steadystate['Reverse'] = ode_likelihoods_steadystate[
'Forward'].rdiv(1)
ode_likelihoods_steadystate['For-Rev'] = ode_likelihoods_steadystate['
Forward'] - ode_likelihoods_steadystate['Reverse']
ode_likelihoods_steadystate['Rxn Probabilities'] = ode_likelihoods_ste
adystate['For-Rev']/np.sum(abs(ode_likelihoods_steadystate['For-Rev'])
)
display(ode_likelihoods_steadystate)
```

	Forward	Reverse	For-Rev	Rxn Probabilities
ME1m	0.000000	0.000000e+00	0.000000	0.000000
ME2m	0.000000	0.000000e+00	0.000000	0.000000
CSm	6.554156	5.290375e-14	6.554156	0.051767
ACONTm	6.703329	1.491725e-01	6.554156	0.051767
ICDHxm	6.582371	2.821428e-02	6.554157	0.051767
AKGDm	6.582372	2.821556e-02	6.554156	0.051767
SUCOASm	6.703335	1.491783e-01	6.554157	0.051767
SUCD1m	6.703332	1.491752e-01	6.554157	0.051767
FUMm	6.703327	1.491710e-01	6.554156	0.051767
MDHm	6.554156	5.290088e-14	6.554156	0.051767
GAPD	7.933921	1.260317e-01	7.807889	0.061669
PGK	7.933929	1.260406e-01	7.807889	0.061669
TPI	4.145176	2.412312e-01	3.903944	0.030834
MDH	0.000000	0.000000e+00	0.000000	0.000000
PEP_Carboxylase	0.000000	0.000000e+00	0.000000	0.000000
PPCK	0.000000	0.000000e+00	0.000000	0.000000
FBA	4.145176	2.412312e-01	3.903944	0.030834

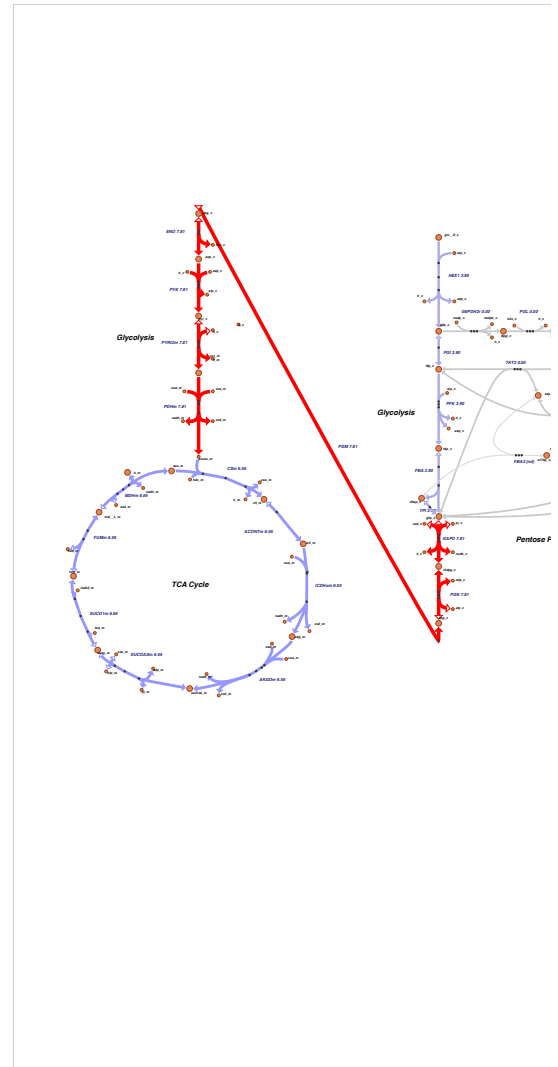
FBP	0.000000	0.000000e+00	0.000000	0.000000
TKT2	0.000000	0.000000e+00	0.000000	0.000000
RPE	0.000000	0.000000e+00	0.000000	0.000000
Xylulokinase	0.000000	0.000000e+00	0.000000	0.000000
PYK_org	0.000000	0.000000e+00	0.000000	0.000000
PYK	7.933929	1.260406e-01	7.807889	0.061669
RPI	0.000000	0.000000e+00	0.000000	0.000000
TKT1	0.000000	0.000000e+00	0.000000	0.000000
TALA	0.000000	0.000000e+00	0.000000	0.000000
PGM	7.933930	1.260410e-01	7.807889	0.061669
ENO	7.933930	1.260410e-01	7.807889	0.061669
GND	0.000000	0.000000e+00	0.000000	0.000000
PGL	0.000000	0.000000e+00	0.000000	0.000000
HEX1	4.145744	2.412103e-01	3.904533	0.030839
PGI	4.145744	2.412112e-01	3.904533	0.030839
G6PDH2r	0.000000	0.000000e+00	0.000000	0.000000
PFK	4.145744	2.412105e-01	3.904533	0.030839
PYRt2m	7.933929	1.260410e-01	7.807888	0.061669
PDHm	7.933923	1.260362e-01	7.807887	0.061669
ICL	0.000000	0.000000e+00	0.000000	0.000000
MAS	0.000000	0.000000e+00	0.000000	0.000000
PYRDC	0.000000	0.000000e+00	0.000000	0.000000
ALDD2y	0.000000	0.000000e+00	0.000000	0.000000
ALCD2X_copy1	0.000000	0.000000e+00	0.000000	0.000000
LactateDehydrogenase	0.000000	0.000000e+00	0.000000	0.000000

Map Net Likelihoods onto Metabolic Pathways

```
In [46]: from escher import Builder

reaction_data = ode_likeliholds_steadystate['For-Rev'].to_dict()
#b = Builder(map_name="iMM904.PPP_TCA_Glycolysis.json",reaction_data=r
eaction_data)
b = Builder(map_name="iMM904.compact_Glycolysis_TCA_PPP.json",reaction
_data=reaction_data)
#b.display_in_browser(menu='all')
b.display_in_notebook(menu='zoom')
```

Out[46]:



Find Active and Variable Metabolites

Some metabolites concentrations are parameters - those that form the boundary conditions such as cofactors, glucose and CO_2 - and others are free variables. Here we find the subset of metabolites that are free variables.

```
In [47]: # Find active reactions
# find chemicals that are in at least one reaction. Includes chemicals
# that are fixed as well as
# variable ones:
S_active = pd.read_table('neurospora_pentose_phos.glycolysis.tca.2_no_
reg.amat',header=0, index_col = 0, quoting=2)
del S_active['forward reaction']

active_metabolites_idx = (S_active != 0).any(axis=0) #any searches dow
n the column
inactive_metabolites_idx = ~active_metabolites_idx

metabolites_status = pd.read_csv('neurospora_pentose_phos.glycolysis.t
ca.2_no_reg.rstrt',delimiter = '\t', index_col=0,skiprows = 2, header=
None,quoting=2,usecols=[0,2])
metabolites_status.rename(columns={2:'Value'},inplace=True)
metabolites_status.index = metabolites_status.index.str.strip()
metabolites_status['Variable?'] = metabolites_status == 'V'
metabolites_status.insert(1,'Active?',active_metabolites_idx.values)
del metabolites_status['Value']

metabolites_status['Variable & Active'] = metabolites_status['Variable
?']&metabolites_status['Active?']
variable_metabolite_idx = list(metabolites_status[metabolites_status['
Variable & Active']==True].index)
variable_metabolite_idx = list(map(str.strip, variable_metabolite_idx)
)
```

Time Derivatives of Metabolite Concentration: $S \cdot \text{Net Odds} = \text{flux}$

This is a demonstration that the product of the stoichiometric matrix S and the net thermodynamic odds are close to zero, which is the steady state condition discussed in the manuscript. Here the stoichiometric matrix $S^{m,n}$ is defined as m reactions by n metabolites, which is the transpose of how it is used in the metabolic modeling literature.

```
In [48]: S = pd.read_table('neurospora_pentose_phos.glycolysis.tca.2_no_reg.mat',header=0, index_col = 0, quoting=2)
del S['forward reaction']

S = S.astype(np.float64)
# S.T.dot(ode_likeliheids_steadystate['For-Rev']) = time derivative of concentrations
#
# 0 at steady state and maximum entropy.
# If boundary conditions prevent maximum entropy, then the flux !=0 for boundary species.
derivatives2 = (S.T.dot(ode_likeliheids_steadystate['For-Rev'])).sort_values()
display(derivatives2[variable_metabolite_idx].sort_values())
```

CITRATE:MITOCHONDRIA	-4.990009e-07
SUCCINYL-COA:MITOCHONDRIA	-2.600002e-07
ISOCITRATE:MITOCHONDRIA	-2.200002e-07
SUCCINATE:MITOCHONDRIA	-9.900094e-08
D-GLYCERALDEHYDE-3-PHOSPHATE:CYTOSOL	-2.457542e-08
BETA-D-GLUCOSE-6-PHOSPHATE:CYTOSOL	-1.108892e-08
2-PHOSPHO-D-GLYCERATE:CYTOSOL	-6.193806e-09
PHOSPHOENOLPYRUVATE:CYTOSOL	-1.598404e-09
(S)-MALATE:MITOCHONDRIA	-9.989307e-10
OXALOACETATE:MITOCHONDRIA	0.000000e+00
GLYCERONE_PHOSPHATE:CYTOSOL	1.098900e-09
D-FRUCTOSE_6-PHOSPHATE:CYTOSOL	5.944056e-08
3-PHOSPHO-D-GLYCERATE:CYTOSOL	7.042957e-08
3-PHOSPHO-D-GLYCEROYL_PHOSPHATE:CYTOSOL	2.501498e-07
2-OXOGLUTARATE:MITOCHONDRIA	2.800002e-07
PYRUVATE:CYTOSOL	5.807193e-07
FUMARATE:MITOCHONDRIA	7.990009e-07
PYRUVATE:MITOCHONDRIA	8.989011e-07
D-FRUCTOSE_1,6-BISPHOSPHATE:CYTOSOL	5.887064e-04
ACETYL-COA:MITOCHONDRIA	1.253731e+00

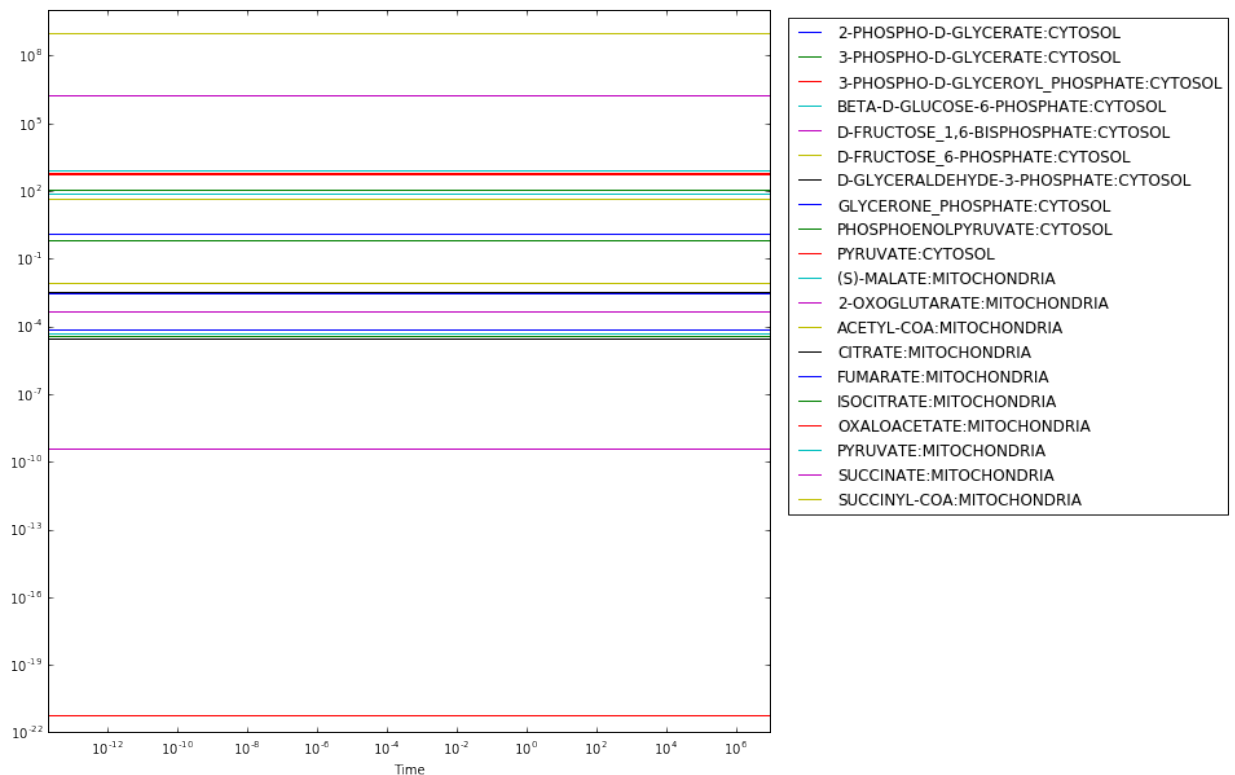
dtype: float64

Analyze Variable Metabolite Levels from Deterministic Simulation

```
In [49]: # Read boltzmann stochastic reaction likelihoods
ode_metabolites = pd.read_table('neurospora_pentose_phos.glycolysis.tca.2_no_reg.ode_concs',header=0,index_col=0)

fig = plt.figure();
plot = ode_metabolites[variable_metabolite_idx].plot(legend=False,logy=True,logx=True,figsize=(10, 10))
fig = plot.get_figure()
fig.savefig('metabolites_no_regulation.png')
plt.legend(bbox_to_anchor=(1.40, 0.90),bbox_transform=plt.gcf().transformFigure)
fig.savefig('metabolites_no_regulation_legend.png',bbox_inches='tight')
)
```

<matplotlib.figure.Figure at 0x105048e48>



```
In [50]: ode_metabolites_steadystate = \
pd.DataFrame(data = ode_metabolites.iloc[-20:,:].mean(),columns=['ODE', 'E'])
ode_metabolites_steadystate.sort_values(by=['ODE'])
```

Out[50]:

	ODE
D-GLUCONO-1,5-LACTONE_6-PHOSPHATE:CYTOSOL	0.000000e+00
LACTATE:CYTOSOL	0.000000e+00
6-PHOSPHO-D-GLUCONATE:CYTOSOL	0.000000e+00
ACETALDEHYDE:CYTOSOL	0.000000e+00

ACETATE:CYTOSOL	0.000000e+00
ETHANOL:CYTOSOL	0.000000e+00
OXALOACETATE:MITOCHONDRIA	5.723803e-22
2-OXOGLUTARATE:MITOCHONDRIA	3.787650e-10
D-ERYTHROSE-4-PHOSPHATE:CYTOSOL	5.149502e-08
D-RIBULOSE-5-PHOSPHATE:CYTOSOL	2.890365e-07
D-RIBOSE-5-PHOSPHATE:CYTOSOL	3.189369e-07
GLYOXYLATE:GLYOXYSOME	1.000000e-06
NADP+:MITOCHONDRIA	2.100000e-06
NADP+:CYTOSOL	2.100000e-06
D-XYLULOSE-5-PHOSPHATE:CYTOSOL	2.320598e-06
D-GLYCERALDEHYDE-3-PHOSPHATE:CYTOSOL	3.084260e-05
ISOCITRATE:MITOCHONDRIA	3.539343e-05
(S)-MALATE:MITOCHONDRIA	4.706638e-05
FUMARATE:MITOCHONDRIA	7.350155e-05
NADH:MITOCHONDRIA	8.300000e-05
NADH:CYTOSOL	8.300000e-05
CO2:MITOCHONDRIA	1.000000e-04
CO2:CYTOSOL	1.000000e-04
NADPH:MITOCHONDRIA	1.200000e-04
NADPH:CYTOSOL	1.200000e-04
SEDOHEPTULOSE_7-PHOSPHATE:CYTOSOL	3.035266e-04
SUCCINATE:MITOCHONDRIA	4.927172e-04
ADP:CYTOSOL	5.600000e-04
ADP:MITOCHONDRIA	5.600000e-04
SUCCINATE:GLYOXYSOME	5.700000e-04
ISOCITRATE:GLYOXYSOME	1.000000e-03
OXALOACETATE:CYTOSOL	1.000000e-03
REDOX1:MITOCHONDRIA	1.000000e-03
REDOX2:MITOCHONDRIA	1.000000e-03
COA:MITOCHONDRIA	1.400000e-03

COA:GLYOXYSOME	1.400000e-03
(S)-MALATE:CYTOSOL	1.700000e-03
(S)-MALATE:GLYOXYSOME	1.700000e-03
BETA-D-GLUCOSE:CYTOSOL	2.000000e-03
NAD+:MITOCHONDRIA	2.600000e-03
NAD+:CYTOSOL	2.600000e-03
GLYCERONE_PHOSPHATE:CYTOSOL	2.808746e-03
ACETYL-COA:GLYOXYSOME	2.863050e-03
CITRATE:MITOCHONDRIA	3.467543e-03
D-XYLULOSE:CYTOSOL	8.800000e-03
SUCCINYL-COA:MITOCHONDRIA	8.866397e-03
ATP:CYTOSOL	9.600000e-03
ATP:MITOCHONDRIA	9.600000e-03
ORTHOPHOSPHATE:CYTOSOL	2.000000e-02
ORTHOPHOSPHATE:MITOCHONDRIA	2.000000e-02
PHOSPHOENOLPYRUVATE:CYTOSOL	6.645813e-01
2-PHOSPHO-D-GLYCERATE:CYTOSOL	1.330016e+00
D-FRUCTOSE_6-PHOSPHATE:CYTOSOL	4.584099e+01
BETA-D-GLUCOSE-6-PHOSPHATE:CYTOSOL	7.607134e+01
3-PHOSPHO-D-GLYCERATE:CYTOSOL	1.138637e+02
3-PHOSPHO-D-GLYCEROYL_PHOSPHATE:CYTOSOL	5.803109e+02
PYRUVATE:CYTOSOL	6.084000e+02
PYRUVATE:MITOCHONDRIA	8.427092e+02
D-FRUCTOSE_1,6-BISPHOSPHATE:CYTOSOL	1.643838e+06
ACETYL-COA:MITOCHONDRIA	1.043645e+09

Infer Regulated Reactions

Regulated reactions are inferred using a loss function defined as the log ratio of the observed values $n_{i(\alpha)}$ to the predicted values $\tilde{n}_{i(\alpha)}$ of the $M(\alpha)$ reaction products of reaction α , $[L\{\alpha\} = \log \prod_{i \in M(\alpha)} \frac{n_{i(\alpha)}}{\tilde{n}_{i(\alpha)}}]$.


```

In [51]: ode_metabolites_steadystate['Expected'] = 1.0e-03

S = pd.read_table('neurospora_pentose_phos.glycolysis.tca.2_no_reg.mat',
header=0, index_col = 0, quoting=2)
P = (S>0)
del P['forward reaction']

P = P.astype(np.float64)
#display(R)
product_concentrations = P.multiply(ode_metabolites_steadystate['ODE'],
axis=1)
product_concentrations[product_concentrations == 0] = 1
product_concentrations_rxns = pd.DataFrame(data = product_concentratio
ns.T.product(), columns=['ODE'])
expect_product_concentrations = P.multiply(ode_metabolites_steadystate
['Expected'],axis=1)
expect_product_concentrations[expect_product_concentrations == 0] = 1
product_concentrations_rxns['Expected'] = (expect_product_concentratio
ns.T).product()

s_regulation = '$L_{reg}$'
product_concentrations_rxns[s_regulation] = \
    np.log(product_concentrations_rxns['ODE']/product_concentrations_r
xns['Expected'])

idx = (ode_likeliheids_steadystate['Reverse'] != 0) & (ode_likeliheids
_steadystate['Forward'] != 0) & \
    (product_concentrations_rxns[s_regulation]>1)
#display(product_concentrations_rxns[product_concentrations_rxns[s_reg
ulation]>1])
display(product_concentrations_rxns[idx].sort_values(by=s_regulation))
product_concentrations_rxns[idx].to_csv(path_or_buf='loss_no_regulatio
n.txt',sep='\t')

```

	ODE	Expected	L_{reg}
reaction title			
CSm	4.854560e-06	1.000000e-06	1.579919
SUCOASm	6.622119e-09	1.000000e-09	1.890415
ENO	6.645813e-01	1.000000e-03	6.499157
PGM	1.330016e+00	1.000000e-03	7.192947
HEX1	4.259995e-02	1.000000e-06	10.659608
PGI	4.584099e+01	1.000000e-03	10.732934
GAPD	4.816581e-02	1.000000e-06	10.782405
PYRt2m	8.427092e+02	1.000000e-03	13.644377
PGK	1.093091e+00	1.000000e-06	13.904520
PYK	5.840640e+00	1.000000e-06	15.580351
PFK	9.205491e+02	1.000000e-06	20.640481
PDHm	8.662250e+00	1.000000e-09	22.882240