The *Escherichia coli* Core Model: Bond Graph Analysis

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October 28, 2019

Contents

Note: this is the EcoliCoreModel.ipynb notebook. The PDF version "The Escherichia coli Core Model: Bond Graph Analysis" is available here.

1 Introduction

The bond graph approach (Oster et al., 1971, 1973; Gawthrop and Crampin, 2014; Gawthrop et al., 2015; Gawthrop and Crampin, 2016, 2017) to modelling biomolecular systems of interest to systems biologists developed independently from the stoichiometric approach (Palsson, 2006, 2011, 2015).

However, the conceptual point of intersection of the two approaches is the fact that the stoichiometric matrix is the modulus of the conceptual multiport transformer linking reactions to species. This was pointed out by (Cellier and Greifeneder, 2009). This means that the two approaches are complementary and each can build on the strengths of the other.

This notebook focuses on building modular models of metabolism and consequent pathway analysis; energetic issues are not explicitly considered here although they are implicit in the bond graph models.

1.1 Strengths of the stoichiometric approach

1. The stoichiometric matrix provides a precise description of the structure of chemical reaction networks.
2. Stoichiometric matrices are available for biologically significant systems including whole-cell models.
3. Powerful linear algebra concepts such as matrix null spaces and singular-value decompositions can be applied.
4. It provides the basis for flux-balance analysis (Orth et al., 2010a).

1.2 Strengths of the bond graph approach

1. Bond graph provide an energy-based approach to modelling.
2. The bond graph model can be analysed for energy flows and efficiency as described by (Gawthrop and Crampin, 2018).
3. The bond graph approach is multi domain and can thus, for example, model electrochemical systems including neurodynamics (Gawthrop et al., 2017) and redox reactions (Gawthrop, 2017a)

1.3 Import some python code

The bond graph analysis uses a number of Python modules:

```
In [1]: import importlib as imp
        import Extract
        import stoich as st
        imp.reload(st)
        ## Stoich conversion
        import stoichBondGraph as stbg
        #imp.reload(stbg)
        import numpy as np
```

```
import BondGraphTools as bgt
## Display (eg disp.SVG(), disp.
import IPython.display as disp
from timeit import default_timer as timer
```

1.4 Combining the two approaches

The key stoichiometric concept of pathways has already been given a bond graph interpretation (Gawthrop, 2017b; Gawthrop and Crampin, 2017)

This note shows how:

1. bond graphs can be created from stoichiometric data using the ecoli core model (Orth et al., 2010b) as an example.
2. bond graphs of subsystems can be extracted from such stoichiometric data
3. pathways can be anaysed and behavior elucidated using appropriate chice of chemostats
4. bond graphs of the extracted subsystems can be recombined in a modular fashion

Much remains to be done in exploiting the combination of the two approaches including:

1. A new look at flux-balance analysis.
2. Further work on energy and efficiency of cellular systems.
3. Using the large stoichiometric models available either directly or extracted from models in SBML or cellML format.

1.5 Missing electrons

It seems that some stoichiometric models (for example the ecoli core model (Orth et al., 2010a)) do not contain information about charges in general and electrons in particular. There appears to be an ambiguity in that that species H sometimes means H^+ and sometimes $H \rightleftharpoons H^+ + e^-$.

1.6 External metabolites

The standard stoichiometric approach is to create open systems from closed systems by adding "dangling reactions" to species which connect to the outside world as external metabolites-- for example: ATP $\rightleftharpoons$. In contrast, the bond graph approacj would declare ATP to be a chemostat. Thus when extracting a bond graph from a stoichiometric model, dangling reactions are deleted and the corresponding species added to a list of chemostats.

Chemostats provide a more flexible approach as they can be created without changing system structure.

2 Extract various modules from the ecoli core model

2.1 Extract full ecoli core model

In this case the ecoli core model is extracted from a spreadsheet describing the model: ecoli_core_model.xlsx. This is modified to remove the "biomass" column and an integer version of the stoichiometric matrix, together with reactions and species is produced.

```
In [2]: imp.reload(Extract)
        sm_real = Extract.extract(quiet=True)
        sm = Extract.Integer(sm_real)
        sm['name'] = 'ecoli_core_model_abg'
```

```
Multiplying reaction CYTBD ( 14 ) by 2.0 to avoid non-integer species O2 ( 55 )
```

2.1.1 Create a bond graph from the stoichiometric matrix, reactions and species.

```
In [3]: stbg.model(sm)
        import ecoli_core_model_abg
```

2.2 Extract TCA part of full model

A list of the reactions of the TCA cycle is provided and the corresponding species and stoichiometric matrix are extracted. Note that the NAD/NADP interconversion reaction NADTRHD is included.

```
In [4]: reaction = ['CS','ACONTA','ACONTB','ICDHYR','AKGDH','SUCOAS',
                    'FRD7',
                    'SUCDI','FUM','MDH',
                'NADTRHD']
        s0 = Extract.choose(sm,reaction=reaction)
        disp.Latex(st.sprintrl(s0,chemformula=True))
```

Out[4]:

$$\mathrm{ACCOA} + \mathrm{H_2O} + \mathrm{OAA} \xrightleftharpoons{\mathrm{CS}} \mathrm{CIT} + \mathrm{COA} + \mathrm{H} \tag{1}$$

$$\mathrm{CIT} \xrightleftharpoons{\mathrm{ACONTA}} \mathrm{ACONC} + \mathrm{H_2O} \tag{2}$$

$$\mathrm{ACONC} + \mathrm{H_2O} \xrightleftharpoons{\mathrm{ACONTB}} \mathrm{ICIT} \tag{3}$$

$$\mathrm{ICIT} + \mathrm{NADP} \xrightleftharpoons{\mathrm{ICDHYR}} \mathrm{AKG} + \mathrm{CO_2} + \mathrm{NADPH} \tag{4}$$

$$\mathrm{AKG} + \mathrm{COA} + \mathrm{NAD} \xrightleftharpoons{\mathrm{AKGDH}} \mathrm{CO_2} + \mathrm{NADH} + \mathrm{SUCCOA} \tag{5}$$

$$\mathrm{ATP} + \mathrm{COA} + \mathrm{SUCC} \xrightleftharpoons{\mathrm{SUCOAS}} \mathrm{ADP} + \mathrm{PI} + \mathrm{SUCCOA} \tag{6}$$

$$\mathrm{FUM} + \mathrm{Q_8H_2} \xrightleftharpoons{\mathrm{FRD_7}} \mathrm{Q_8} + \mathrm{SUCC} \tag{7}$$

$$\mathrm{Q_8} + \mathrm{SUCC} \xrightleftharpoons{\mathrm{SUCDI}} \mathrm{FUM} + \mathrm{Q_8H_2} \tag{8}$$

$$\mathrm{FUM} + \mathrm{H_2O} \xrightleftharpoons{\mathrm{FUM}} \mathrm{MALL} \tag{9}$$

$$\mathrm{MALL} + \mathrm{NAD} \xrightleftharpoons{\mathrm{MDH}} \mathrm{H} + \mathrm{NADH} + \mathrm{OAA} \tag{10}$$

$$\mathrm{NAD} + \mathrm{NADPH} \xrightleftharpoons{\mathrm{NADTRHD}} \mathrm{NADH} + \mathrm{NADP} \tag{11}$$

2.2.1 Create bond graph and recreate stoichiometry

The bond graph TCA_abg is created and written to TCA_abg.py from whence it can me imported. The stoichiometric matrix generated from TCA_abg.model() is compared with that extracted from the ecoli core model to check that all is working correctely.

```
In [5]: s0['name'] = 'TCA_abg'
        stbg.model(s0)
        import TCA_abg
        imp.reload(TCA_abg)
        s = st.stoich(TCA_abg.model(),quiet=True)
        err = np.linalg.norm(s['N']-s0['N'])
        print("Error:",err)
```

```
Error: 0.0
```

```
In [6]: #bgt.draw(TCA_abg.model())
```

2.2.2 Pathway analysis

Thee species corresponding to ATP hydrolysis, NAD/NADH, NADP/NADPH and Q8/Q8H2 (ubiquone, but maybe FAD, I think) are set as the basic chemostats in the list chemostats0.

The overall reaction of the TCA cycle converts ACCOA to COA and CO_2 (Garrett and Grisham, 2017), so these three species are also set as chemostats.

```
In [7]: print(st.sprintp(s))
        chemostats0 = ['ADP', 'ATP', 'H2O', 'NAD', 'NADH', 'PI','H'
                       ,'Q8','Q8H2']
        sc0 = st.statify(s,chemostats=chemostats0)
        print(st.sprintp(sc0))
        chemostats = ['ACCOA','COA','CO2']
        chemostats.extend(chemostats0)
        sc = st.statify(s,chemostats=chemostats)
        print(st.sprintp(sc))
```

```
1 pathways
0:  + FRD7 + SUCDI

1 pathways
0:  + FRD7 + SUCDI

2 pathways
0:  + FRD7 + SUCDI
1:  + CS + ACONTA + ACONTB + ICDHYR + AKGDH - SUCOAS - FRD7 + FUM + MDH + NADTRHD
```

With only the basic chemostats, there is one internal cycle. This performs no conversions and so the reaction is empty.

```
In [8]: sp0 = st.path(s,sc0)
        disp.Latex(st.sprintrl(sp0,chemformula=True,split=10))
```

Out[8]:

$$\xrightleftharpoons{pr_1} \tag{12}$$

When the chemostats ['ACCOA','COA','CO2'] are added, the entire TCA cycle appears as a pathway and the overall reaction is

```
In [9]: sp = st.path(s,sc)
        disp.Latex(st.sprintrl(sp,chemformula=True,split=10))
```

Out[9]:

$$\xrightleftharpoons{pr_1} \tag{13}$$

$$\mathrm{ACCOA + ADP + 2\,H_2O + 3\,NAD + PI + Q_8} \xrightleftharpoons{pr_2} \mathrm{ATP + 2\,CO_2 + COA + 2\,H + 3\,NADH + Q_8H_2} \tag{14}$$

Pathway reactions:

1. A null cycle (shared with closed system)
2. Pathway from ACCOA to CO2

2.3 Include Pyruvate (Pyr) reactions: PDH and PFL

To take this example further, include the two reactions converting pyruvate to ACCOA.

```
In [10]: reaction.extend(['PDH','PFL'])
         s0 = Extract.choose(sm,reaction=reaction)
         disp.Latex(st.sprintrl(s0,chemformula=True))
```

Out[10]:

$$\mathrm{ACCOA + H_2O + OAA} \xrightleftharpoons{CS} \mathrm{CIT + COA + H} \tag{15}$$

$$\mathrm{CIT} \xrightleftharpoons{ACONTA} \mathrm{ACONC + H_2O} \tag{16}$$

$$\mathrm{ACONC + H_2O} \xrightleftharpoons{ACONTB} \mathrm{ICIT} \tag{17}$$

$$\mathrm{ICIT + NADP} \xrightleftharpoons{ICDHYR} \mathrm{AKG + CO_2 + NADPH} \tag{18}$$

$$\mathrm{AKG + COA + NAD} \xrightleftharpoons{AKGDH} \mathrm{CO_2 + NADH + SUCCOA} \tag{19}$$

$$\mathrm{ATP + COA + SUCC} \xrightleftharpoons{SUCOAS} \mathrm{ADP + PI + SUCCOA} \tag{20}$$

$$\mathrm{FUM + Q_8H_2} \xrightleftharpoons{FRD_7} \mathrm{Q_8 + SUCC} \tag{21}$$

$$\mathrm{Q_8 + SUCC} \xrightleftharpoons{SUCDI} \mathrm{FUM + Q_8H_2} \tag{22}$$

$$\mathrm{FUM + H_2O} \xrightleftharpoons{FUM} \mathrm{MALL} \tag{23}$$

$$\mathrm{MALL + NAD} \xrightleftharpoons{MDH} \mathrm{H + NADH + OAA} \tag{24}$$

$$\text{NAD} + \text{NADPH} \xrightleftharpoons{\text{NADTRHD}} \text{NADH} + \text{NADP} \tag{25}$$

$$\text{COA} + \text{NAD} + \text{PYR} \xrightleftharpoons{\text{PDH}} \text{ACCOA} + \text{CO}_2 + \text{NADH} \tag{26}$$

$$\text{COA} + \text{PYR} \xrightleftharpoons{\text{PFL}} \text{ACCOA} + \text{FOR} \tag{27}$$

2.3.1 Create bond graph and recreate stoichiometry

```
In [11]: s0['name'] = 'PyrTCA_abg'
         stbg.model(s0)
         import PyrTCA_abg
         imp.reload(PyrTCA_abg)
         s = st.stoich(PyrTCA_abg.model(),quiet=True)
         err = np.linalg.norm(s['N']-s0['N'])
         print("Error:",err)
```

```
Error: 0.0
```

2.3.2 Pathway analysis

The relevant chemostats are now the substrate PYR and the product CO_2 together with the reactions ATP hydrolysis, NAD/NADH, NADP/NADPH and Q8/Q8H2 nf the additional product FOR

```
In [12]: print(st.sprintp(s))
         chemostats0 = ['ADP', 'ATP', 'H2O', 'NAD', 'NADH', 'PI','NADP', 'NADPH','H'
                        ,'Q8','Q8H2','FOR']
         sc0 = st.statify(s,chemostats=chemostats0)
         print(st.sprintp(sc0))
         chemostats = ['PYR','CO2']
         chemostats.extend(chemostats0)
         sc = st.statify(s,chemostats=chemostats)
         print(st.sprintp(sc))
```

```
1 pathways
0:  + FRD7 + SUCDI

2 pathways
0:  + FRD7 + SUCDI
1:  + NADTRHD

4 pathways
0:  + FRD7 + SUCDI
1:  + NADTRHD
2:  + CS + ACONTA + ACONTB + ICDHYR + AKGDH - SUCOAS - FRD7 + FUM + MDH + PDH
3:  + CS + ACONTA + ACONTB + ICDHYR + AKGDH - SUCOAS - FRD7 + FUM + MDH + PFL
```

```
In [13]: sp0 = st.path(s,sc0)
         disp.Latex(st.sprintrl(sp0,chemformula=True,split=10))
```

Out[13]:

$$\xrightleftharpoons{\mathrm{pr}_1} \tag{28}$$

```
In [14]: sp = st.path(s,sc)
         disp.Latex(st.sprintrl(sp,chemformula=True,split=10))
```

Out[14]:

$$\xrightleftharpoons{\mathrm{pr}_1} \tag{29}$$

$$\mathrm{ADP} + 2\,\mathrm{H_2O} + 3\,\mathrm{NAD} + \mathrm{NADP} + \mathrm{PI} + \mathrm{PYR} + \mathrm{Q_8} \xrightleftharpoons{\mathrm{pr}_2} \mathrm{ATP} + 3\,\mathrm{CO_2} + 2\,\mathrm{H} + 3\,\mathrm{NADH} + \mathrm{NADPH} + \mathrm{Q_8H_2} \tag{30}$$

$$\mathrm{ADP} + 2\,\mathrm{H_2O} + 2\,\mathrm{NAD} + \mathrm{NADP} + \mathrm{PI} + \mathrm{PYR} + \mathrm{Q_8} \xrightleftharpoons{\mathrm{pr}_3} \mathrm{ATP} + 2\,\mathrm{CO_2} + \mathrm{FOR} + 2\,\mathrm{H} + 2\,\mathrm{NADH} + \mathrm{NADPH} + \mathrm{Q} \tag{31}$$

Pathway reactions:

1. A null cycle (shared with closed system)
2. Pathway from PYR to CO2 via reaction PDH
3. Pathway from PYR to CO2 via reaction PFL

2.4 Extract the Electron Transport Chain (ETC)

```
In [15]: reaction = ['NADH16','CYTBD']
         s0 = Extract.choose(sm,reaction=reaction)
         disp.Latex(st.sprintrl(s0,chemformula=True))
```

Out[15]:

$$4\,\mathrm{H} + \mathrm{NADH} + \mathrm{Q_8} \xrightleftharpoons{\mathrm{NADH_{16}}} 3\,\mathrm{H_E} + \mathrm{NAD} + \mathrm{Q_8H_2} \tag{32}$$

$$4\,\mathrm{H} + \mathrm{O_2} + 2\,\mathrm{Q_8H_2} \xrightleftharpoons{\mathrm{CYTBD}} 2\,\mathrm{H_2O} + 4\,\mathrm{H_E} + 2\,\mathrm{Q_8} \tag{33}$$

```
In [16]: s0['name'] = 'ETC_abg'
         stbg.model(s0)
         import ETC_abg
         imp.reload(ETC_abg)
         s = st.stoich(ETC_abg.model(),quiet=True)
         err = np.linalg.norm(s['N']-s0['N'])
         print("Error:",err)
         s['reaction'] = s0['reaction']
```

```
Error: 0.0
```

2.4.1 Pathway analysis

```
In [17]: print(st.sprintp(s))
         chemostats = ['NADH','NAD','O2','H2O','H','H_E']
         sc = st.statify(s,chemostats=chemostats)
         print(st.sprintp(sc))
```

```
0 pathways

1 pathways
0:  + 2 NADH16 + CYTBD
```

```
In [18]: sp = st.path(s,sc)
         disp.Latex(st.sprintrl(sp,chemformula=True))
```

Out[18]:

$$12\,\mathrm{H} + 2\,\mathrm{NADH} + \mathrm{O_2} \xrightleftharpoons{\mathrm{pr_1}} 2\,\mathrm{H_2O} + 10\,\mathrm{H_E} + 2\,\mathrm{NAD} \tag{34}$$

2.5 Extract ATPase

```
In [19]: reaction = ['ATPS4R']
         s0 = Extract.choose(sm,reaction=reaction)
         disp.Latex(st.sprintrl(s0,chemformula=True))
```

Out[19]:

$$\mathrm{ADP} + 4\,\mathrm{H_E} + \mathrm{PI} \xrightleftharpoons{\mathrm{ATPS_4R}} \mathrm{ATP} + 3\,\mathrm{H} + \mathrm{H_2O} \tag{35}$$

```
In [20]: s0['name'] = 'ATP_abg'
         stbg.model(s0)
         import ATP_abg
         imp.reload(ATP_abg)
         s = st.stoich(ATP_abg.model(),quiet=True)
         err = np.linalg.norm(s['N']-s0['N'])
         print("Error:",err)
         s['reaction'] = s0['reaction']
```

```
Error: 0.0
```

2.5.1 Pathway analysis

```
In [21]: print(st.sprintp(s))
         chemostats = ['H','H_E','ATP','ADP','PI','H2O']
         sc = st.statify(s,chemostats=chemostats)
         print(st.sprintp(sc,removeSingle=False))
```

```
0 pathways

1 pathways
0:  + ATPS4R
```

```
In [22]: sp = st.path(s,sc,removeSingle=False)
         disp.Latex(st.sprintrl(sp,chemformula=True))
```

Out[22]:

$$\mathrm{ADP} + 4\,\mathrm{H_E} + \mathrm{PI} \xrightleftharpoons{\mathrm{pr_1}} \mathrm{ATP} + 3\,\mathrm{H} + \mathrm{H_2O} \tag{36}$$

2.6 Extract Glycolysis

```
In [23]: reaction = ['GLCPTS','PGI','PFK','FBP','FBA','TPI','GAPD','PGK','PGM','ENO','PYK']
         s0 = Extract.choose(sm,reaction=reaction)
         disp.Latex(st.sprintrl(s0,chemformula=True))
```

Out[23]:

$$\mathrm{GLCD_E} + \mathrm{PEP} \xrightleftharpoons{\mathrm{GLCPTS}} \mathrm{G_6P} + \mathrm{PYR} \tag{37}$$

$$\mathrm{G_6P} \xrightleftharpoons{\mathrm{PGI}} \mathrm{F_6P} \tag{38}$$

$$\mathrm{ATP} + \mathrm{F_6P} \xrightleftharpoons{\mathrm{PFK}} \mathrm{ADP} + \mathrm{FDP} + \mathrm{H} \tag{39}$$

$$\mathrm{FDP} + \mathrm{H_2O} \xrightleftharpoons{\mathrm{FBP}} \mathrm{F_6P} + \mathrm{PI} \tag{40}$$

$$\mathrm{FDP} \xrightleftharpoons{\mathrm{FBA}} \mathrm{DHAP} + \mathrm{G_3P} \tag{41}$$

$$\mathrm{DHAP} \xrightleftharpoons{\mathrm{TPI}} \mathrm{G_3P} \tag{42}$$

$$\mathrm{G_3P} + \mathrm{NAD} + \mathrm{PI} \xrightleftharpoons{\mathrm{GAPD}} {}_{13}\mathrm{DPG} + \mathrm{H} + \mathrm{NADH} \tag{43}$$

$${}_{3}\mathrm{PG} + \mathrm{ATP} \xrightleftharpoons{\mathrm{PGK}} {}_{13}\mathrm{DPG} + \mathrm{ADP} \tag{44}$$

$${}_{2}\mathrm{PG} \xrightleftharpoons{\mathrm{PGM}} {}_{3}\mathrm{PG} \tag{45}$$

$${}_{2}\mathrm{PG} \xrightleftharpoons{\mathrm{ENO}} \mathrm{H_2O} + \mathrm{PEP} \tag{46}$$

$$\mathrm{ADP} + \mathrm{H} + \mathrm{PEP} \xrightleftharpoons{\mathrm{PYK}} \mathrm{ATP} + \mathrm{PYR} \tag{47}$$

```
In [24]: s0['name'] = 'GLY_abg'
         stbg.model(s0)
         import GLY_abg
         imp.reload(GLY_abg)
         s = st.stoich(GLY_abg.model(),quiet=True)
         err = np.linalg.norm(s['N']-s0['N'])
         print("Error:",err)
```

```
Error: 0.0
```

2.6.1 Pathway analysis

```
In [25]: chemostats0 = ['ADP','ATP','H2O','PI','H']
         sc0 = st.statify(s,chemostats=chemostats0)
         chemostats = ['GLCD_E','PYR','NAD','NADH']
         chemostats.extend(chemostats0)
         sc = st.statify(s,chemostats=chemostats)
         print(st.sprintp(sc))
         sp = st.path(s,sc)
         disp.Latex(st.sprintrl(sp,chemformula=True))
```

```
2 pathways
0:  + PFK + FBP
1:  + GLCPTS + PGI + PFK + FBA + TPI + 2 GAPD - 2 PGK - 2 PGM + 2 ENO + PYK
```

Out[25]:

$$\mathrm{ATP} + \mathrm{H_2O} \underset{}{\overset{\mathrm{pr_1}}{\rightleftharpoons}} \mathrm{ADP} + \mathrm{H} + \mathrm{PI} \tag{48}$$

$$2\,\mathrm{ADP} + \mathrm{GLCD_E} + 2\,\mathrm{NAD} + 2\,\mathrm{PI} \overset{\mathrm{pr_2}}{\rightleftharpoons} 2\,\mathrm{ATP} + 2\,\mathrm{H} + 2\,\mathrm{H_2O} + 2\,\mathrm{NADH} + 2\,\mathrm{PYR} \tag{49}$$

3 Modularity: the Glycolysis/TCA network

To examine the glycolysis/TCA network, it is convenient and informative to take a modular approach: the glycolysis network and the TCA network are extracted as separate subsystems and then combined. The example shows two approaches to combining the subsystems:

1. combining the stoichiometric matrices
2. combining the bond graphs.

3.1 Create composite model

```
In [26]: GlyTCA = bgt.new(name='GlyTCA')    # Create system
         Gly = GLY_abg.model()
         TCA = PyrTCA_abg.model()
         GlyTCA.add(Gly,TCA)
```

3.1.1 Unify common species - stoichiometric approach

PYR is produced by Gly and consumed by TCA. ATP, ADP, PI, H, NAD, NADH , H2O are common to both modules.

```
In [27]: imp.reload(st)
         common = ['PYR','ATP','ADP','PI','H','NAD','NADH','H2O']
         st.unify(s,commonSpecies=common)
```

3.1.2 Pathway analysis

```
In [28]: chemostats0 = ['ADP', 'ATP', 'H2O', 'NAD', 'NADH', 'PI','H'
                        ,'Q8','Q8H2','FOR']
         chemostats = ['GLCD_E','CO2']
         chemostats.extend(chemostats0)
         sc = st.statify(s,chemostats=chemostats)
         print(st.sprintp(sc))
```

```
Chemostat CO2 is not a model species
Chemostat Q8 is not a model species
Chemostat Q8H2 is not a model species
Chemostat FOR is not a model species
1 pathways
0:  + PFK + FBP
```

```
In [29]: sp = st.path(s,sc)
         disp.Latex(st.sprintrl(sp,chemformula=True))
```

Out[29]:

$$\mathrm{ATP} + \mathrm{H_2O} \overset{\mathrm{pr_1}}{\rightleftharpoons} \mathrm{ADP} + \mathrm{H} + \mathrm{PI} \tag{50}$$

Pathway reactions:

1. A futile cycle
2. A null cycle
3. Pathway from GLCD_E to CO2 via reaction PDH
4. Pathway from GLCD_E to CO2 via reaction PFL

3.1.3 Unify common species - bond graph approach

This version creates a bond graph model of the unified system by explicitly replacing C components by ports in the subsystems, creating new C components and connecting via 0 junctions and ports.

```
In [30]: import modular
         modular.unify(GlyTCA,common=common,quiet=True)
```

```
In [31]: ##bgt.draw(GlyTCA)
```

Pathway analysis

```
In [32]: s = st.stoich(GlyTCA,quiet=True)
         disp.Latex(st.sprintrl(s,chemformula=True))
```

Out[32]:

$$\mathrm{GLCD_E} + \mathrm{PEP} \xrightleftharpoons{\mathrm{GLCPTS}} \mathrm{G_6P} + \mathrm{PYR} \tag{51}$$

$$\mathrm{G_6P} \xrightleftharpoons{\mathrm{PGI}} \mathrm{F_6P} \tag{52}$$

$$\mathrm{F_6P} + \mathrm{ATP} \xrightleftharpoons{\mathrm{PFK}} \mathrm{FDP} + \mathrm{ADP} + \mathrm{H} \tag{53}$$

$$\mathrm{FDP} + \mathrm{H_2O} \xrightleftharpoons{\mathrm{FBP}} \mathrm{F_6P} + \mathrm{PI} \tag{54}$$

$$\mathrm{FDP} \xrightleftharpoons{\mathrm{FBA}} \mathrm{DHAP} + \mathrm{G_3P} \tag{55}$$

$$\mathrm{DHAP} \xrightleftharpoons{\mathrm{TPI}} \mathrm{G_3P} \tag{56}$$

$$\mathrm{G_3P} + \mathrm{PI} + \mathrm{NAD} \xrightleftharpoons{\mathrm{GAPD}} {}_{13}\mathrm{DPG} + \mathrm{H} + \mathrm{NADH} \tag{57}$$

$${}_{3}\mathrm{PG} + \mathrm{ATP} \xrightleftharpoons{\mathrm{PGK}} {}_{13}\mathrm{DPG} + \mathrm{ADP} \tag{58}$$

$${}_{2}\mathrm{PG} \xrightleftharpoons{\mathrm{PGM}} {}_{3}\mathrm{PG} \tag{59}$$

$${}_{2}\mathrm{PG} \xrightleftharpoons{\mathrm{ENO}} \mathrm{PEP} + \mathrm{H_2O} \tag{60}$$

$$\mathrm{PEP} + \mathrm{ADP} + \mathrm{H} \xrightleftharpoons{\mathrm{PYK}} \mathrm{PYR} + \mathrm{ATP} \tag{61}$$

$$\mathrm{ACCOA} + \mathrm{OAA} + \mathrm{H_2O} \xrightleftharpoons{\mathrm{CS}} \mathrm{CIT} + \mathrm{COA} + \mathrm{H} \tag{62}$$

$$\mathrm{CIT} \xrightleftharpoons{\mathrm{ACONTA}} \mathrm{ACONC} + \mathrm{H_2O} \tag{63}$$

$$\mathrm{ACONC} + \mathrm{H_2O} \xrightleftharpoons{\mathrm{ACONTB}} \mathrm{ICIT} \tag{64}$$

$$\mathrm{ICIT} + \mathrm{NADP} \xrightleftharpoons{\mathrm{ICDHYR}} \mathrm{AKG} + \mathrm{CO_2} + \mathrm{NADPH} \tag{65}$$

$$\mathrm{AKG} + \mathrm{COA} + \mathrm{NAD} \xrightleftharpoons{\mathrm{AKGDH}} \mathrm{CO_2} + \mathrm{SUCCOA} + \mathrm{NADH} \tag{66}$$

$$\mathrm{COA} + \mathrm{SUCC} + \mathrm{ATP} \xrightleftharpoons{\mathrm{SUCOAS}} \mathrm{SUCCOA} + \mathrm{ADP} + \mathrm{PI} \tag{67}$$

$$\mathrm{FUM} + \mathrm{Q_8H_2} \xrightleftharpoons{\mathrm{FRD_7}} \mathrm{Q_8} + \mathrm{SUCC} \tag{68}$$

$$\mathrm{Q_8} + \mathrm{SUCC} \xrightleftharpoons{\mathrm{SUCDI}} \mathrm{FUM} + \mathrm{Q_8H_2} \tag{69}$$

$$\mathrm{FUM} + \mathrm{H_2O} \xrightleftharpoons{\mathrm{FUM}} \mathrm{MALL} \tag{70}$$

$$\mathrm{MALL} + \mathrm{NAD} \xrightleftharpoons{\mathrm{MDH}} \mathrm{OAA} + \mathrm{H} + \mathrm{NADH} \tag{71}$$

$$\mathrm{NADPH} + \mathrm{NAD} \xrightleftharpoons{\mathrm{NADTRHD}} \mathrm{NADP} + \mathrm{NADH} \tag{72}$$

$$\mathrm{COA} + \mathrm{PYR} + \mathrm{NAD} \xrightleftharpoons{\mathrm{PDH}} \mathrm{ACCOA} + \mathrm{CO_2} + \mathrm{NADH} \tag{73}$$

$$\mathrm{COA} + \mathrm{PYR} \xrightleftharpoons{\mathrm{PFL}} \mathrm{ACCOA} + \mathrm{FOR} \tag{74}$$

In [33]:
```
sc = st.statify(s,chemostats=chemostats)
print(st.sprintp(sc))
```

```
4 pathways
0:  + PFK + FBP
```

```
1:  + FRD7 + SUCDI
2:  + GLCPTS + PGI + PFK + FBA + TPI + 2 GAPD - 2 PGK - 2 PGM + 2 ENO + PYK + 2 CS + 2 ACONTA +
3:  + GLCPTS + PGI + PFK + FBA + TPI + 2 GAPD - 2 PGK - 2 PGM + 2 ENO + PYK + 2 CS + 2 ACONTA +
```

```
In [34]: sp = st.path(s,sc)
         disp.Latex(st.sprintrl(sp,chemformula=True))
```

Out[34]:

$$\mathrm{ATP} + \mathrm{H_2O} \overset{\mathrm{pr_1}}{\rightleftharpoons} \mathrm{ADP} + \mathrm{PI} + \mathrm{H} \tag{75}$$

$$\overset{\mathrm{pr_2}}{\rightleftharpoons} \tag{76}$$

$$\mathrm{GLCD_E} + 2\,\mathrm{Q_8} + 4\,\mathrm{ADP} + 4\,\mathrm{PI} + 10\,\mathrm{NAD} + 2\,\mathrm{H_2O} \overset{\mathrm{pr_3}}{\rightleftharpoons} 6\,\mathrm{CO_2} + 2\,\mathrm{Q_8H_2} + 4\,\mathrm{ATP} + 6\,\mathrm{H} + 10\,\mathrm{NADH} \tag{77}$$

$$\mathrm{GLCD_E} + 2\,\mathrm{Q_8} + 4\,\mathrm{ADP} + 4\,\mathrm{PI} + 8\,\mathrm{NAD} + 2\,\mathrm{H_2O} \overset{\mathrm{pr_4}}{\rightleftharpoons} 4\,\mathrm{CO_2} + 2\,\mathrm{FOR} + 2\,\mathrm{Q_8H_2} + 4\,\mathrm{ATP} + 6\,\mathrm{H} + 8\,\mathrm{NADH} \tag{78}$$

Pathway reactions are the same as before:

1. A futile cycle
2. A null cycle
3. Pathway from GLCD_E to CO2 via reaction PDH
4. Pathway from GLCD_E to CO2 via reaction PFL

4 Modularity: metabolism

The above methods are brought together to generate the metabolic pathways by combining the modules describing: Glycolysis, the TCA cycle, the Electron Transport Chain and ATPase. Once again, the overall bond graph is created by comineing moduels and unifying common species.

```
In [35]: Met = bgt.new(name='Met')    # Create system
         Gly = GLY_abg.model()
         TCA = PyrTCA_abg.model()
         ETC = ETC_abg.model()
         ATP = ATP_abg.model()
         Met.add(Gly,TCA,ETC,ATP)
```

```
In [36]: ## Unify species common to modules
         common = ['PYR','ATP','ADP','PI','H','H_E','NAD','NADH','H2O','Q8','Q8H2']
         modular.unify(Met,common=common,quiet=True)
```

4.1 Pathway analysis

```
In [37]: s = st.stoich(Met,quiet=True)
         disp.Latex(st.sprintrl(s,chemformula=True))
```

Out[37]:

$$\mathrm{GLCD_E + PEP \xrightleftharpoons{GLCPTS} G_6P + PYR} \tag{79}$$
$$\mathrm{G_6P \xrightleftharpoons{PGI} F_6P} \tag{80}$$
$$\mathrm{F_6P + ATP \xrightleftharpoons{PFK} FDP + ADP + H} \tag{81}$$
$$\mathrm{FDP + H_2O \xrightleftharpoons{FBP} F_6P + PI} \tag{82}$$
$$\mathrm{FDP \xrightleftharpoons{FBA} DHAP + G_3P} \tag{83}$$
$$\mathrm{DHAP \xrightleftharpoons{TPI} G_3P} \tag{84}$$
$$\mathrm{G_3P + PI + NAD \xrightleftharpoons{GAPD} {}_{13}DPG + H + NADH} \tag{85}$$
$$\mathrm{{}_3PG + ATP \xrightleftharpoons{PGK} {}_{13}DPG + ADP} \tag{86}$$
$$\mathrm{{}_2PG \xrightleftharpoons{PGM} {}_3PG} \tag{87}$$
$$\mathrm{{}_2PG \xrightleftharpoons{ENO} PEP + H_2O} \tag{88}$$

$$\mathrm{PEP + ADP + H \xrightleftharpoons{PYK} PYR + ATP} \tag{89}$$
$$\mathrm{ACCOA + OAA + H_2O \xrightleftharpoons{CS} CIT + COA + H} \tag{90}$$
$$\mathrm{CIT \xrightleftharpoons{ACONTA} ACONC + H_2O} \tag{91}$$
$$\mathrm{ACONC + H_2O \xrightleftharpoons{ACONTB} ICIT} \tag{92}$$
$$\mathrm{ICIT + NADP \xrightleftharpoons{ICDHYR} AKG + CO_2 + NADPH} \tag{93}$$
$$\mathrm{AKG + COA + NAD \xrightleftharpoons{AKGDH} CO_2 + SUCCOA + NADH} \tag{94}$$
$$\mathrm{COA + SUCC + ATP \xrightleftharpoons{SUCOAS} SUCCOA + ADP + PI} \tag{95}$$
$$\mathrm{FUM + Q_8H_2 \xrightleftharpoons{FRD_7} SUCC + Q_8} \tag{96}$$
$$\mathrm{SUCC + Q_8 \xrightleftharpoons{SUCDI} FUM + Q_8H_2} \tag{97}$$
$$\mathrm{FUM + H_2O \xrightleftharpoons{FUM} MALL} \tag{98}$$

$$\mathrm{MALL + NAD \xrightleftharpoons{MDH} OAA + H + NADH} \tag{99}$$
$$\mathrm{NADPH + NAD \xrightleftharpoons{NADTRHD} NADP + NADH} \tag{100}$$
$$\mathrm{COA + PYR + NAD \xrightleftharpoons{PDH} ACCOA + CO_2 + NADH} \tag{101}$$
$$\mathrm{COA + PYR \xrightleftharpoons{PFL} ACCOA + FOR} \tag{102}$$
$$\mathrm{4\,H + NADH + Q_8 \xrightleftharpoons{NADH_{16}} 3\,H_E + NAD + Q_8H_2} \tag{103}$$
$$\mathrm{O_2 + 4\,H + 2\,Q_8H_2 \xrightleftharpoons{CYTBD} 4\,H_E + 2\,H_2O + 2\,Q_8} \tag{104}$$
$$\mathrm{ADP + PI + 4\,H_E \xrightleftharpoons{ATPS_4R} ATP + 3\,H + H_2O} \tag{105}$$

```
In [38]: imp.reload(st)
         chemostats0 = ['ADP','ATP','H2O','PI','H']
         chemostats = ['GLCD_E','CO2','O2']
         chemostats.extend(chemostats0)
         print(chemostats)
         sc = st.statify(s,chemostats=chemostats)
         print(st.sprintp(sc))
```

```
['GLCD_E', 'CO2', 'O2', 'ADP', 'ATP', 'H2O', 'PI', 'H']
3 pathways
0:  + PFK + FBP
1:  + FRD7 + SUCDI
2:  + 2 GLCPTS + 2 PGI + 2 PFK + 2 FBA + 2 TPI + 4 GAPD - 4 PGK - 4 PGM + 4 ENO + 2 PYK + 4 CS +
```

```
In [39]: sp = st.path(s,sc)
         disp.Latex(st.sprintrl(sp,chemformula=True))
```

Out[39]:

$$\mathrm{ATP} + \mathrm{H_2O} \overset{\mathrm{pr}_1}{\rightleftharpoons} \mathrm{ADP} + \mathrm{PI} + \mathrm{H} \tag{106}$$

$$\overset{\mathrm{pr}_2}{\rightleftharpoons} \tag{107}$$

$$2\,\mathrm{GLCD_E} + 12\,\mathrm{O_2} + 35\,\mathrm{ADP} + 35\,\mathrm{PI} + 35\,\mathrm{H} \overset{\mathrm{pr}_3}{\rightleftharpoons} 12\,\mathrm{CO_2} + 35\,\mathrm{ATP} + 47\,\mathrm{H_2O} \tag{108}$$

There are three pathways:

1. A futile cycle
2. A null cycle
3. Each GLCD_E, combined with $6\,O_2$, reverses ATP hydrolysis ($\mathrm{ATP} + \mathrm{H_2O} \rightleftharpoons \mathrm{ADP} + \mathrm{PI} + \mathrm{H^+}$) to give 17.5 ATP molecules with an additional $6\,H_2O$ and $6\,CO_2$; this is the value quoted by (Palsson, 2015).

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