

The *Escherichia coli* Core Model: Bond Graph Analysis

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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., 2010b).

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

## Modular bond graphs
# import modularBondGraph as mbg

```

```

In [2]: import BondGraphTools as bgt
import numpy as np
import IPython.display as disp

## Stoichiometric analysis
import stoich as st

## Export stoichiometry as bond graph
import stoichBondGraph as stbg

## Modular bond graphs
import modularBondGraph as mbg

## Extract stoichiometry from spreadsheet
import Extract

```

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., 2010a) as an example.
2. bond graphs of subsystems can be extracted from such stoichiometric data
3. pathways can be analysed and behavior elucidated using appropriate choice 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., 2010b)) 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: $\text{ATP} \rightleftharpoons \cdot$. In contrast, the bond graph approach 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 [3]: ##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 02 (55)

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

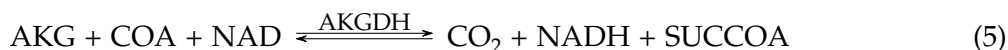
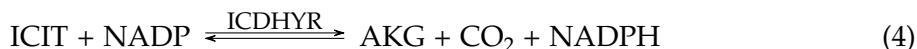
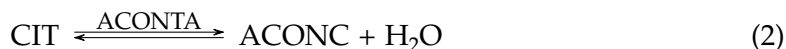
```
In [4]: stbg.model(sm)
        import ecoli_core_model_abg
```

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 [5]: 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 [5]:



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 be 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 correctly.

```
In [6]: 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 [7]: #bgt.draw(TCA_abg.model())
```

2.2.2 Pathway analysis

Three 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 CO₂ (Garrett and Grisham, 2017), so these three species are also set as chemostats.

```
In [8]: 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 [9]: sp0 = st.path(s, sc0)
        disp.Latex(st.sprintrl(sp0, chemformula=True, split=10))

```

Out [9]:



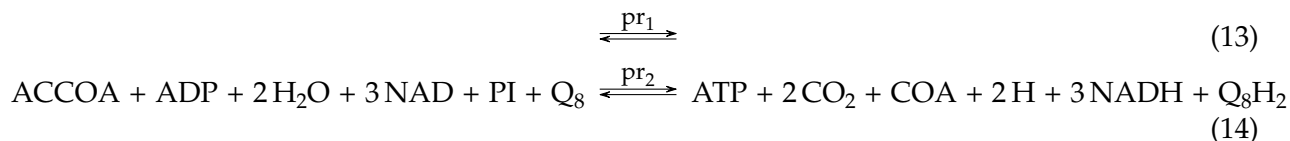
When the chemostats ['ACCOA', 'COA', 'CO2'] are added, the entire TCA cycle appears as a pathway and the overall reaction is

```

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

```

Out [10]:



Pathway reactions:

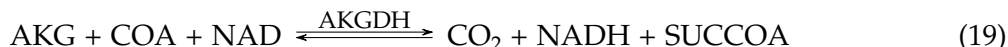
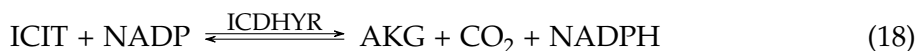
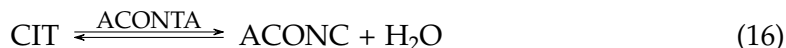
1. A null cycle (shared with closed system)
2. Pathway from ACCOA to CO2

2.3 Include Pyruvate (Pyr) reactions: PDH and PFL

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

```
In [11]: reaction.extend(['PDH','PFL'])
         s0 = Extract.choose(sm,reaction=reaction)
         disp.Latex(st.sprintrl(s0,chemformula=True))
```

Out[11]:



2.3.1 Create bond graph and recreate stoichiometry

```
In [12]: 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 CO₂ together with the reactions ATP hydrolysis, NAD/NADH, NADP/NADPH and Q8/Q8H₂ and the additional product FOR

```
In [13]: 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

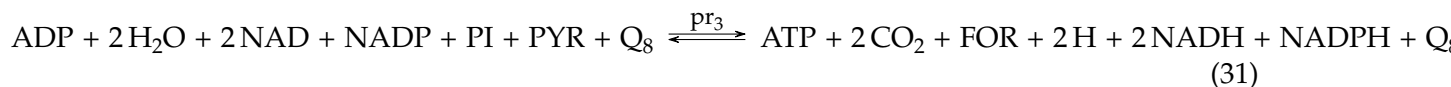
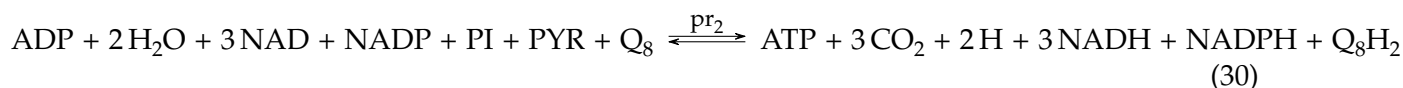
```
In [14]: sp0 = st.path(s, sc0)
          disp.Latex(st.sprintrl(sp0, chemformula=True, split=10))
```

Out[14]:



```
In [15]: sp = st.path(s, sc)
          disp.Latex(st.sprintrl(sp, chemformula=True, split=10))
```

Out[15]:



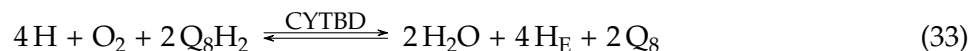
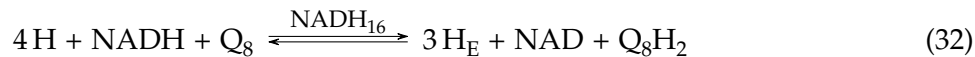
Pathway reactions:

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

Out[16]:



```
In [17]: 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 [18]: print(st.sprintp(s))
         chemostats = ['NADH', 'NAD', 'O2', 'H2O', 'H', 'H_E']
         sc = st.statify(s, chemostats=chemostats)
         print(st.sprintp(sc))
```

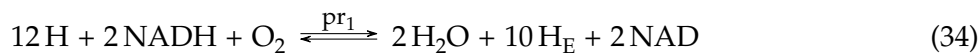
0 pathways

1 pathways

0: + 2 NADH16 + CYTBD

```
In [19]: sp = st.path(s, sc)
         disp.Latex(st.sprintrl(sp, chemformula=True))
```

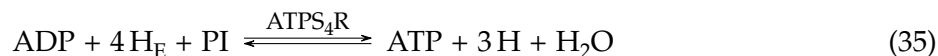
Out[19]:



2.5 Extract ATPase

```
In [20]: reaction = ['ATPS4R']
         s0 = Extract.choose(sm, reaction=reaction)
         disp.Latex(st.sprintrl(s0, chemformula=True))
```

Out[20]:



```
In [21]: 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 [22]: print(st.sprintp(s))
         chemostats = ['H', 'H_E', 'ATP', 'ADP', 'PI', 'H2O']
         sc = st.statify(s, chemostats=chemostats)
         print(st.sprintp(sc, removeSingle=False))
```

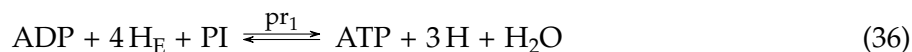
0 pathways

1 pathways

0: + ATPS4R

```
In [23]: sp = st.path(s, sc, removeSingle=False)
         disp.Latex(st.sprintrl(sp, chemformula=True))
```

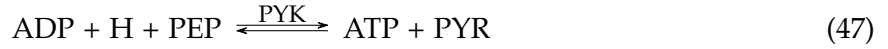
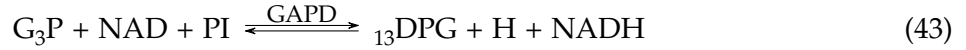
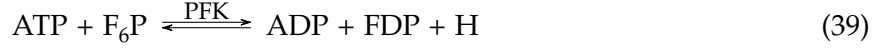
Out[23]:



2.6 Extract Glycolysis

```
In [24]: 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 [24] :



```
In [25]: 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 [26]: 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 [26] :



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 [27]: 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 [28]: ##imp.reload(st)
        common = ['PYR', 'ATP', 'ADP', 'PI', 'H', 'NAD', 'NADH', 'H2O']
        st.unify(s,commonSpecies=common)
```

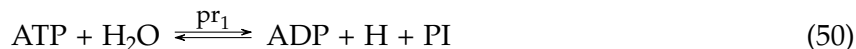
3.1.2 Pathway analysis

```
In [29]: 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 [30]: sp = st.path(s,sc)
         disp.Latex(st.sprintrl(sp,chemformula=True))
```

Out [30]:



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.

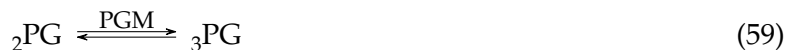
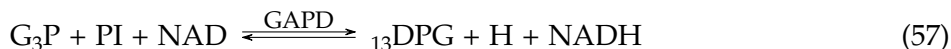
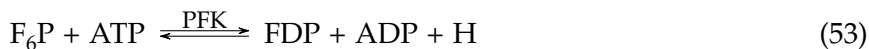
```
In [31]: mbg.unify(GlyTCA,common=common,quiet=True)
```

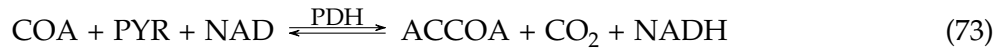
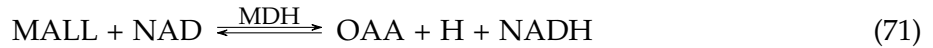
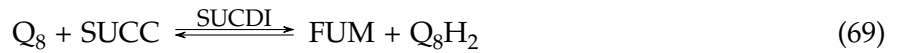
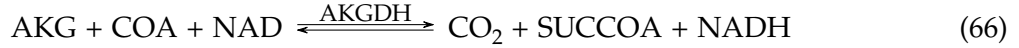
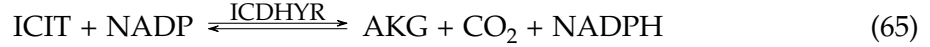
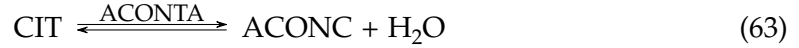
```
In [32]: ##bgt.draw(GlyTCA)
```

Pathway analysis

```
In [33]: s = st.stoich(GlyTCA,quiet=True)
         disp.Latex(st.sprintrl(s,chemformula=True))
```

Out [33]:





```
In [34]: sc = st.statify(s,chemostats=chemostats)
         print(st.sprintp(sc))
```

4 pathways

0: + PFK + FBP

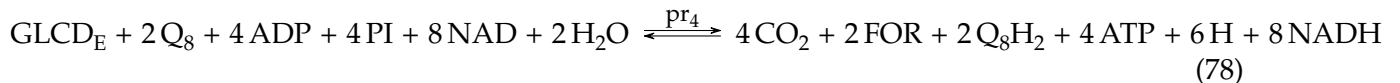
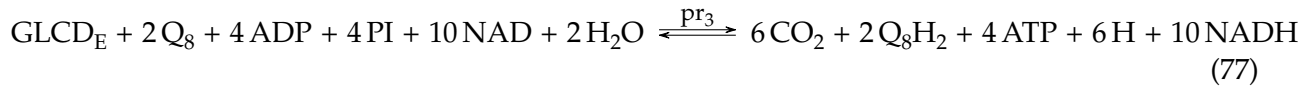
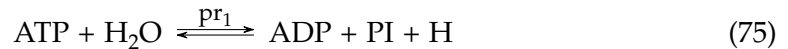
1: + FRD7 + SUCDI

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

Out [35]:



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 combining modules and unifying common species.

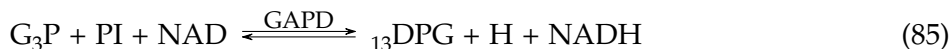
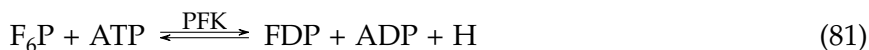
```
In [36]: Met = bgt.new(name='Met')    # Create system
        Gly = GLY_abg.model()
        TCA = PyrTCA_abg.model()
        ETC = ETC_abg.model()
        ATP = ATP_abg.model()
        Met.add(Gly,TCA,ETC,ATP)

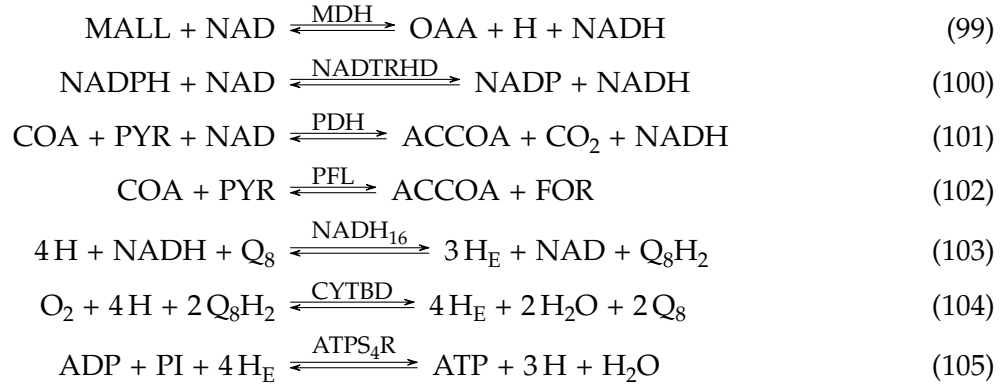
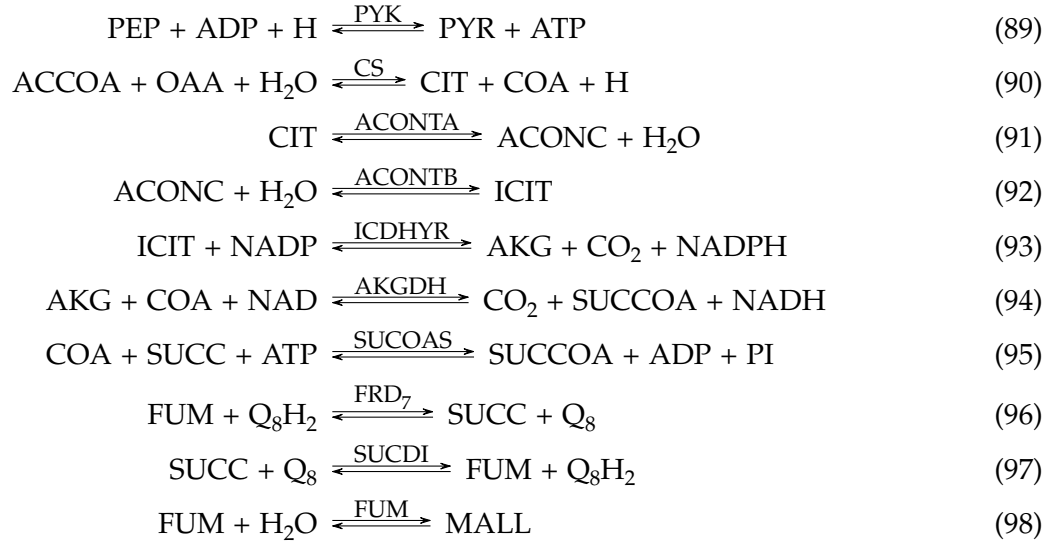
In [37]: ## Unify species common to modules
        common = ['PYR','ATP','ADP','PI','H','H_E','NAD','NADH','H2O','Q8','Q8H2']
        mbg.unify(Met,common=common,quiet=True)
```

4.1 Pathway analysis

```
In [38]: s = st.stoich(Met,quiet=True)
        disp.Latex(st.sprintrl(s,chemformula=True))
```

Out [38]:





In [39]: `##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 +

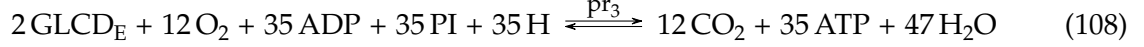
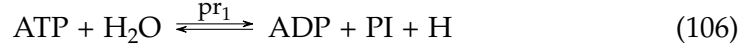
In [40]: `sp = st.path(s, sc)`

```

disp.Latex(st.sprintrl(sp, chemformula=True))

```


Out [40] :



There are three pathways:

1. A futile cycle
2. A null cycle
3. Each GLCD_E, combined with 6 O₂, reverses ATP hydrolysis (ATP + H₂O \rightleftharpoons ADP + PI + H⁺) to give 17.5 ATP molecules with an additional 6 H₂O and 6 CO₂; this is the value quoted by (Palsson, 2015).

References

- F.E. Cellier and J. Greifeneder. Modeling chemical reactions in modelica by use of chemo-bonds. In *Proceedings 7th Modelica Conference*, Como, Italy, September 2009.
- Reginald H. Garrett and Charles M. Grisham. *Biochemistry*. Cengage Learning, Boston, MA, 6th edition, 2017.
- P. J. Gawthrop. Bond graph modeling of chemiosmotic biomolecular energy transduction. *IEEE Transactions on NanoBioscience*, 16(3):177–188, April 2017a. ISSN 1536-1241. doi:[10.1109/TNB.2017.2674683](https://doi.org/10.1109/TNB.2017.2674683). Available at arXiv:1611.04264.
- P. J. Gawthrop and E. J. Crampin. Modular bond-graph modelling and analysis of biomolecular systems. *IET Systems Biology*, 10(5):187–201, October 2016. ISSN 1751-8849. doi:[10.1049/iet-syb.2015.0083](https://doi.org/10.1049/iet-syb.2015.0083). Available at arXiv:1511.06482.
- P. J. Gawthrop, I. Siekmann, T. Kameneva, S. Saha, M. R. Ibbotson, and E. J. Crampin. Bond graph modelling of chemoelectrical energy transduction. *IET Systems Biology*, 11(5):127–138, 2017. ISSN 1751-8849. doi:[10.1049/iet-syb.2017.0006](https://doi.org/10.1049/iet-syb.2017.0006). Available at arXiv:1512.00956.
- Peter J. Gawthrop. Bond-graph modelling and causal analysis of biomolecular systems. In Wolfgang Borutzky, editor, *Bond Graphs for Modelling, Control and Fault Diagnosis of Engineering Systems*, pages 587–623. Springer International Publishing, Berlin, 2017b. ISBN 978-3-319-47434-2. doi:[10.1007/978-3-319-47434-2_16](https://doi.org/10.1007/978-3-319-47434-2_16).
- Peter J. Gawthrop and Edmund J. Crampin. Energy-based analysis of biochemical cycles using bond graphs. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Science*, 470(2171):1–25, 2014. doi:[10.1098/rspa.2014.0459](https://doi.org/10.1098/rspa.2014.0459). Available at arXiv:1406.2447.
- Peter J. Gawthrop and Edmund J. Crampin. Energy-based analysis of biomolecular pathways. *Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences*, 473(2202), 2017. ISSN 1364-5021. doi:[10.1098/rspa.2016.0825](https://doi.org/10.1098/rspa.2016.0825). Available at arXiv:1611.02332.
- Peter J. Gawthrop and Edmund J. Crampin. Biomolecular system energetics. In *Proceedings of the 13th International Conference on Bond Graph Modeling (ICBGM'18)*, Bordeaux, 2018. Society for Computer Simulation. Available at arXiv:1803.09231.

- Peter J. Gawthrop, Joseph Cursons, and Edmund J. Crampin. Hierarchical bond graph modelling of biochemical networks. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 471(2184):1–23, 2015. ISSN 1364-5021. doi:[10.1098/rspa.2015.0642](https://doi.org/10.1098/rspa.2015.0642). Available at arXiv:1503.01814.
- J. Orth, R. Fleming, and B. Palsson. Reconstruction and use of microbial metabolic networks: the core escherichia coli metabolic model as an educational guide. *EcoSal Plus*, 2010a. doi:[10.1128/ecosalplus.10.2.1](https://doi.org/10.1128/ecosalplus.10.2.1).
- Jeffrey D. Orth, Ines Thiele, and Bernhard O. Palsson. What is flux balance analysis? *Nat Biotech*, 28:245–248, March 2010b. ISSN 1087-0156. doi:[10.1038/nbt.1614](https://doi.org/10.1038/nbt.1614).
- George Oster, Alan Perelson, and Aharon Katchalsky. Network thermodynamics. *Nature*, 234: 393–399, December 1971. doi:[10.1038/234393a0](https://doi.org/10.1038/234393a0).
- George F. Oster, Alan S. Perelson, and Aharon Katchalsky. Network thermodynamics: dynamic modelling of biophysical systems. *Quarterly Reviews of Biophysics*, 6(01):1–134, 1973. doi:[10.1017/S0033583500000081](https://doi.org/10.1017/S0033583500000081).
- Bernhard Palsson. *Systems biology: properties of reconstructed networks*. Cambridge University Press, 2006. ISBN 0521859034.
- Bernhard Palsson. *Systems Biology: Simulation of Dynamic Network States*. Cambridge University Press, 2011.
- Bernhard Palsson. *Systems Biology: Constraint-Based Reconstruction and Analysis*. Cambridge University Press, Cambridge, 2015.