

# The *Escherichia coli* Core Model: Modular Bond Graph Analysis

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*Note: this is the EcoliCoreModel.ipynb notebook. The PDF version "The Escherichia coli Core Model: Modular Bond Graph Analysis" is available [here](#).*

## 1 Introduction

The Network Thermodynamics/Bond Graph approach of (Oster et al., 1971, 1973) extended by (Gawthrop and Crampin, 2014, 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 based on the Escherichia coli Core Model (Orth et al., 2010a); 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), redox reactions (Gawthrop, 2017a) and transporters (Pan et al., 2019).
4. The bond graph approach is modular: subsystems can be connected using energy ports (Gawthrop et al., 2015; Gawthrop and Crampin, 2016).
5. The chemostat concept (Gawthrop and Crampin, 2016) gives a convenient and flexible way of turning a closed system into an open system and analysing the concomitant pathway structure.
6. [BondGraphTools](#) provide a symbolic basis for describing and analysing bondgraphs within Python.

### 1.3 Import some python code

The bond graph analysis uses a number of Python modules:

```

In [1]: 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 a CobraPy model
import CobraExtract as Extract

## Control outputs
quiet = True
chemformula = True

```

## 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 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:  $\text{ATP} \rightleftharpoons \text{.}$ . 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.

In the following discussion, chemostats are added as appropriate to illustrate the various pathways.

## 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 the CobraPy model: 'textbook'. This model corresponds to that discussed in the the textbook (Palsson, 2015). An integer version of the stoichiometric matrix, together with reactions and species is produced. Note that the reaction labeled 'Biomass\_Ecoli\_core' is not a reaction but is associated with the optimisation procedure - it can be ignored in this notebook.

Names of reactions and species are converted to upper case and the *c* (cytosol) subscript removed for clarity.

```
In [2]: sm = Extract.extract(Remove=['_C', '__'], quiet=quiet)
```

Extracting stoichiometric matrix from: textbook

Cobra Model name: e\_coli\_core BondGraphTools name: e\_coli\_core\_abg

Extract.Integer only handles one non-integer per reaction

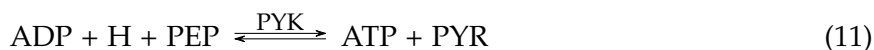
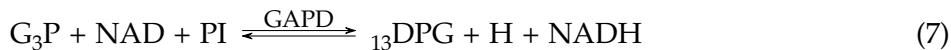
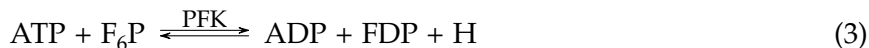
Multiplying reaction BIOMASS\_ECOLIORE ( 12 ) by 0.6684491978609626 to avoid non-integer species

Multiplying reaction CYTBD ( 15 ) by 2.0 to avoid non-integer species 02 ( 55 )

### 2.2 Extract Glycolysis

```
In [3]: 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 [3]:



```
In [4]: s0['name'] = 'GLY_abg'
        stbg.model(s0)
        import GLY_abg
        s = st.stoich(GLY_abg.model(),quiet=True)
        ## Sanity check
        err = np.linalg.norm(s['N']-s0['N'])
        print("Error:",err)
```

Error: 0.0

### 2.2.1 Pathway analysis

```
In [5]: 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 [5]:



Pathway reactions:

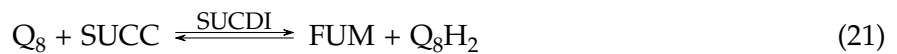
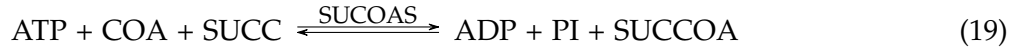
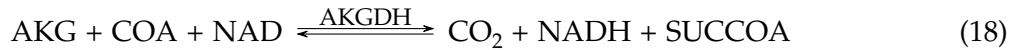
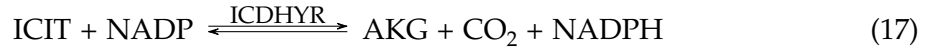
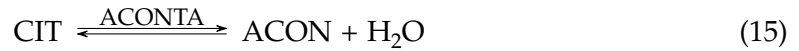
- A futile cycle consuming ATP
- A pathway from GLCD<sub>E</sub> to PYR

### 2.3 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 [6]: 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 [6] :



### 2.3.1 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 [7]: s0['name'] = 'TCA_abg'
        stbg.model(s0)
        import TCA_abg
        s = st.stoich(TCA_abg.model(), quiet=True)

        ## Sanity check
        err = np.linalg.norm(s['N'] - s0['N'])
        print("Error:", err)
```

Error: 0.0

### 2.3.2 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 CO<sub>2</sub> (Garrett and Grisham, 2017), so these three species are also set as chemostats.

```
In [8]: print('No chemostats')
        print(st.sprintp(s))
```

```

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

```

No chemostats

1 pathways

0: + FRD7 + SUCDI

Basic chemostats ['ADP', 'ATP', 'H2O', 'NAD', 'NADH', 'PI', 'H', 'Q8', 'Q8H2']

1 pathways

0: + FRD7 + SUCDI

Chemostats ['ACCOA', 'COA', 'CO2', 'ADP', 'ATP', 'H2O', 'NAD', 'NADH', 'PI', 'H', 'Q8', 'Q8H2']

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]:



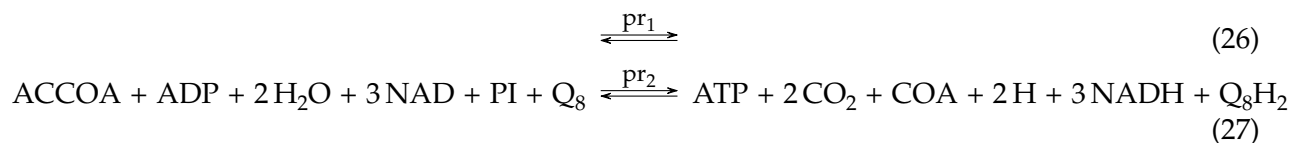
When the chemostats ['ACCOA', 'COA', 'CO2'] are added, the entire TCA cycle appears as a pathway and the overall reaction is

```

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

```

Out[10]:



Pathway reactions:

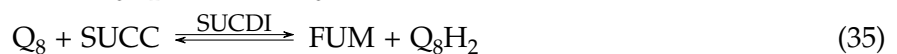
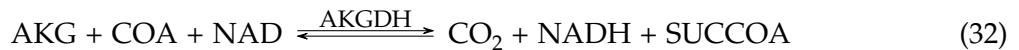
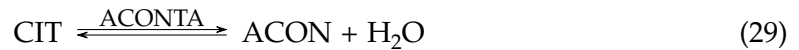
- A null cycle (shared with closed system)
- Pathway from ACCOA to CO<sub>2</sub>

## 2.4 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]:



### 2.4.1 Create bond graph and recreate stoichiometry

```
In [12]: s0['name'] = 'PyrTCA_abg'
         stbg.model(s0)
         import PyrTCA_abg
         s = st.stoich(PyrTCA_abg.model(), quiet=True)

         ## Sanity check
         err = np.linalg.norm(s['N'] - s0['N'])
         print("Error:", err)
```

Error: 0.0



## 2.4.2 Pathway analysis

The relevant chemostats are now the substrate PYR and the product CO<sub>2</sub> together with the reactions ATP hydrolysis, NAD/NADH, NADP/NADPH and Q8/Q8H<sub>2</sub> and the additional product FOR

```
In [13]: print('No chemostats')
          print(st.sprintp(s,removeSingle=True))
          chemostats0 = ['ADP', 'ATP', 'H2O', 'NAD', 'NADH', 'PI', 'NADP', 'NADPH', 'H',
                        , 'Q8', 'Q8H2', 'FOR']
          sc0 = st.statify(s,chemostats=chemostats0)
          print('Basic chemostats',chemostats0)
          print(st.sprintp(sc0,removeSingle=True))
          chemostats = ['PYR', 'CO2']
          chemostats.extend(chemostats0)
          sc = st.statify(s,chemostats=chemostats)
          print('Chemostats',chemostats)
          print(st.sprintp(sc,removeSingle=True))
```

No chemostats

1 non-unit pathways

0: + FRD7 + SUCDI

Basic chemostats ['ADP', 'ATP', 'H2O', 'NAD', 'NADH', 'PI', 'NADP', 'NADPH', 'H', 'Q8', 'Q8H2',

1 non-unit pathways

0: + FRD7 + SUCDI

Chemostats ['PYR', 'CO2', 'ADP', 'ATP', 'H2O', 'NAD', 'NADH', 'PI', 'NADP', 'NADPH', 'H', 'Q8',

3 non-unit pathways

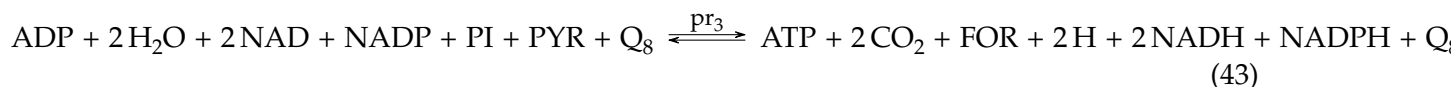
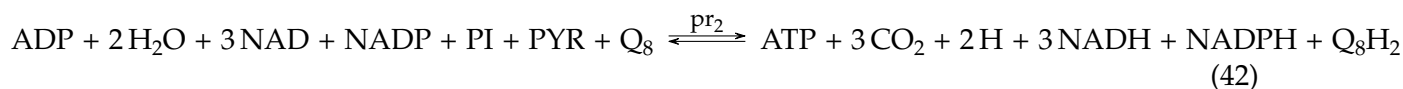
0: + FRD7 + SUCDI

1: + CS + ACONTA + ACONTB + ICDHYR + AKGDH - SUCOAS - FRD7 + FUM + MDH + PDH

2: + CS + ACONTA + ACONTB + ICDHYR + AKGDH - SUCOAS - FRD7 + FUM + MDH + PFL

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

Out[14]:



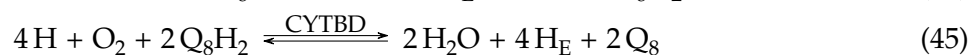
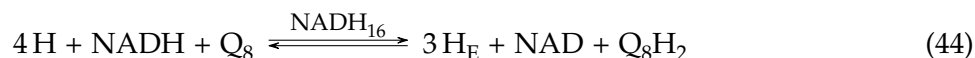
Pathway reactions:

- A null cycle (shared with closed system)
- Pathway from PYR to CO<sub>2</sub> via reaction PDH
- Pathway from PYR to CO<sub>2</sub> via reaction PFL

## 2.5 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]:



```
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

### 2.5.1 Pathway analysis

```
In [17]: print('No chemostats')
         print(st.sprintp(s))
         chemostats = ['NADH', 'NAD', 'O2', 'H2O', 'H', 'H_E']
         sc = st.statify(s, chemostats=chemostats)
         print('Chemostats', chemostats)
         print(st.sprintp(sc))
```

No chemostats

0 pathways

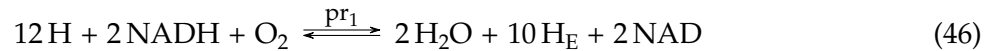
Chemostats ['NADH', 'NAD', 'O2', 'H2O', 'H', 'H\_E']

1 pathways

0: + 2 NADH16 + CYTBD

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

Out [18]:



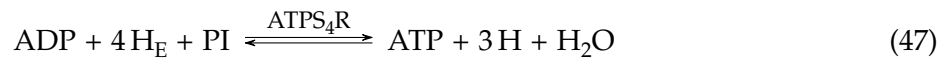
Pathway reactions:

- Pathway from NADH and O<sub>2</sub> to NAD and H<sub>2</sub>O pumping 10 protons

## 2.6 Extract ATPase

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

Out [19]:



```
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

### 2.6.1 Pathway analysis

```
In [21]: print('No chemostats')
         print(st.sprintp(s))
         chemostats = ['H', 'H_E', 'ATP', 'ADP', 'PI', 'H2O']
         sc = st.statify(s, chemostats=chemostats)
         print('Chemostats', chemostats)
         print(st.sprintp(sc, removeSingle=False))
```

No chemostats

0 pathways

Chemostats ['H', 'H\_E', 'ATP', 'ADP', 'PI', 'H2O']

1 pathways

0: + ATPS4R

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

Out [22]:



Pathway reactions:

- Pathway from ADP to ATP pumped by 3 protons.

### 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 [23]: 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

PYR is produced by Gly and consumed by TCA. ATP, ADP, PI, H, NAD, NADH, H<sub>2</sub>O are common to both modules.

```
In [24]: common = ['PYR', 'ATP', 'ADP', 'PI', 'H', 'NAD', 'NADH', 'H2O']
         mbg.unify(GlyTCA,common=common,quiet=quiet)
```

```
In [25]: ## Stoichiometry of unified module
         s = st.stoich(GlyTCA,quiet=True)
         #disp.Latex(st.sprintrl(s,chemformula=True))
```

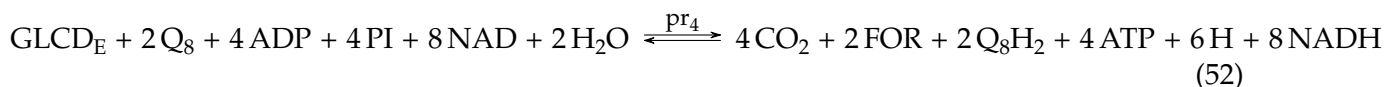
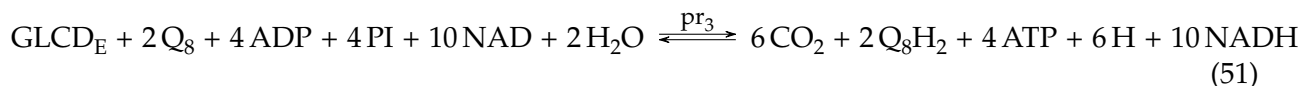
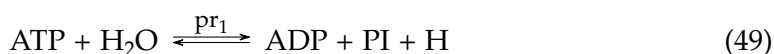
##### 3.1.2 Pathway analysis

```
In [26]: 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('Chemostats',chemostats)
         print(st.sprintp(sc))
```

```
Chemostats ['GLCD_E', 'CO2', 'ADP', 'ATP', 'H2O', 'NAD', 'NADH', 'PI', 'H', 'Q8', 'Q8H2', 'FOR']
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 [27]: sp = st.path(s,sc)
         disp.Latex(st.sprintrl(sp,chemformula=True))
```

Out[27]:



Pathway reactions:

- A futile cycle
- A null cycle
- Pathway from GLCD\_E to CO2 via reaction PDH
- 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.

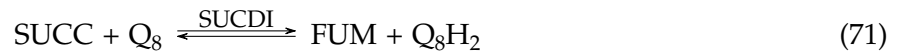
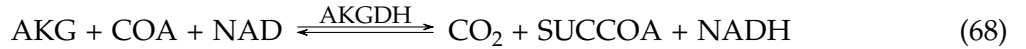
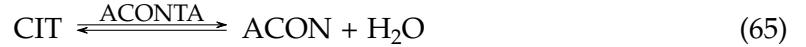
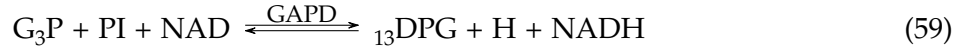
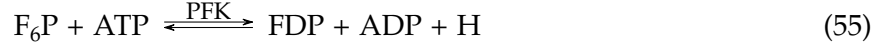
```
In [28]: Met = bgt.new(name='Met')    # Create system
         Gly = GLY_abg.model()
         TCA = PyrTCA_abg.model()
         ETC = ETC_abg.model()
         ATP = ATP_abg.model()
         Met.add(Gly,TCA,ETC,ATP)

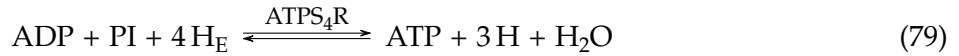
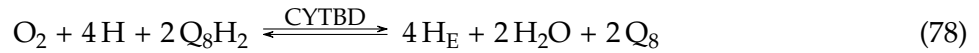
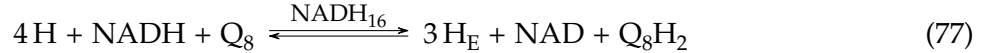
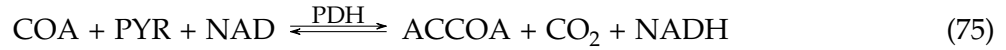
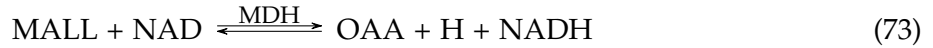
In [29]: ## Unify species common to modules
         common = ['PYR','ATP','ADP','PI','H','H_E','NAD','NADH','H2O','Q8','Q8H2']
         mbg.unify(Met,common=common,quiet=True)
```

## 4.1 Pathway analysis

In [30]: `s = st.stoich(Met,quiet=True)`  
`disp.Latex(st.sprintrl(s,chemformula=True))`

Out [30]:





```
In [31]: chemostats0 = ['ADP', 'ATP', 'H2O', 'PI', 'H']
chemostats = ['GLCD_E', 'CO2', 'O2']
chemostats.extend(chemostats0)
sc = st.statify(s, chemostats=chemostats)
print('Chemostats', chemostats)
print(st.sprintp(sc))
```

Chemostats ['GLCD\_E', 'CO2', 'O2', 'ADP', 'ATP', 'H2O', 'PI', 'H']

3 pathways

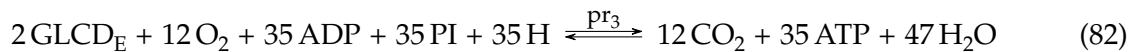
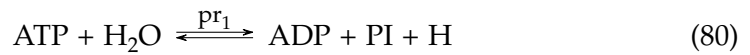
0: + PFK + FBP

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

Out [32]:



There are three pathways:

- A futile cycle
- A null cycle
- Each GLCD\_E, combined with 6 O<sub>2</sub>, reverses ATP hydrolysis (ATP + H<sub>2</sub>O ⇌ ADP + PI + H<sup>+</sup>) to give 17.5 ATP molecules with an additional 6 H<sub>2</sub>O and 6 CO<sub>2</sub>; 17.5 ATP is the value quoted by (Palsson, 2015).

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