Physically-Plausible Parameters

Peter Gawthrop. *peter.gawthrop@unimelb.edu.au*

June 7, 2021

Contents

1 Introduction 1
1.1 Setup modules . . . 2
1.2 Quadratic programming QP. . . . 3

2 Conversion factor 4

3 Extract Model 4
3.1 Extract stoichiometry . . . 4
3.2 Extract reaction potentials Φ and deduce plausible species potentials ϕ. . . . 6
3.3 Extracted reactions and reaction potentials . . . 8

4 Deduce Pathway Flows 9

5 Reaction constants (modified mass-action) and Michaelis-Menten 10
5.1 Normalise data . . . 12
5.2 Show computed reaction flows . . . 12
5.3 Show computed chemostat flows . . . 14
5.4 Show pathway flows . . . 15

6 Species constants 15

7 Simulation 16
7.1 Set up parameters . . . 16
7.2 Set up chemostats and flowstats . . . 17
7.3 Time unit . . . 17
7.4 Simulation . . . 17

8 Michaelis-Menten formulation 25
8.1 Show results . . . 25
8.2 Bond graph model of Enzyme Catalysed Reaction (RE) . . . 25
8.3 Replace Re components by RE . . . 27
8.4 Set up parameters . . . 28
8.5 Simulate . . . 29
8.6 Plot ratios . . . 31

1 Introduction

This note illustrates an approach to fitting the parameters of a bond graph model to experimental data. Insofar as the parameters are associated with a bond graph, they are *physically-plausible* Gawthrop et al. (2020).

The approach uses a bond-graph derived from a stoichiometric model of *e.coli* Orth et al. (2010) (using a method described elsewhere Gawthrop (2020)) combined with experimental values of *reaction potential*, *reaction flux* and *species concentration* from the literature Park et al. (2016).

1.1 Setup modules

[1]:
```
## Paths
NeedPath=False
if NeedPath:
    import sys
    sys.path += ['/usr/lib/python3/dist-packages']
```

[2]:
```
## Maths library
import numpy as np
import scipy

## BG tools
import BondGraphTools as bgt

## SVG bond graph
import svgBondGraph as sbg

## BG stoichiometric utilities
import stoich as st

## Modular bond graphs
import modularBondGraph as mbg

## Stoichiometric conversion
import CobraExtract as Extract
import stoichBondGraph as stbg

## Potentials
import phiData

## Faraday constant
import scipy.constants as con
F = con.physical_constants['Faraday constant'][0]

## Display
import IPython.display as disp

## Plotting
import matplotlib.pyplot as plt


import copy

## Allow output from within functions
```

```
from IPython.core.interactiveshell import InteractiveShell
InteractiveShell.ast_node_interactivity = "all"

import importlib as imp

quiet = True
SaveFig = False
showMu=True
```

1.2 Quadratic programming QP.

$$\text{minimise } \frac{1}{2}x^T P x + q^T x \tag{1}$$
$$\text{subject to } Gx \leq h \tag{2}$$
$$\text{and } Ax = b \tag{3}$$

In the case considered here, there is no equality constraint and

$$x = \hat{\phi} \tag{4}$$
$$P = NN^T + \mu I_{n_X \times n_X} \tag{5}$$
$$q = (N\Phi)^T \tag{6}$$
$$G = N^T \tag{7}$$
$$h = -\Phi_{min} \tag{8}$$

$\mu > 0$ is required to give a convex QP: in essence it turns a non-unique solution for ϕ into a minimum norm solution.

[3]:
```
## Quadratic programming stuff.
import quadprog

## Function from https://scaron.info/blog/quadratic-programming-in-python.html
def quadprog_solve_qp(P, q, G=None, h=None, A=None, b=None):
    qp_G = .5 * (P + P.T)   # make sure P is symmetric
    qp_a = -q
    if A is not None:
        qp_C = -np.vstack([A, G]).T
        qp_b = -np.hstack([b, h])
        meq = A.shape[0]
    else:  # no equality constraint
        if G is None:
            qp_C = None
            qp_b = None
        else:
            qp_C = -G.T
            qp_b = -h
        meq = 0
    return quadprog.solve_qp(qp_G, qp_a, qp_C, qp_b, meq)[0]

## Function to compute phi from Phi subject to Phi>positive number
## NN Reduced N corresponding to known Phi
def quadsolve_phi(N0,N1,Phi0,Phi_min=0.0,mu=1e-10):

    (n_X,n_V) = N1.shape
    P = 1.0*N0@(N0.T) + mu*np.eye(n_X)
    q = (N0@Phi0).T
    G = 1.0*N1.T
    h = -Phi_min*np.ones((n_V))
    phi = quadprog_solve_qp(P, q, G=G, h=h)

    return phi
```

2 Conversion factor

[4]:
```
Factor = st.F()/1e6
print(f'To convert from kJ/mol to mV, divide by {1/Factor:4.3}')
```

```
To convert from kJ/mol to mV, divide by 10.4
```

3 Extract Model

This example uses the Glycolysis and Pentose Phosphate pathways.

Notes:

- Reactions RPI, PGK and PGM are reversed to correspond to positive flows.
- The resultant stoichiometric matrix N relates reaction flows (f) to species flows ($\dot{x}$):

$$\dot{x} = Nf \tag{9}$$

3.1 Extract stoichiometry

[5]:
```
sm = Extract.extract(cobraname='textbook',Remove=['_C','__' ],
negReaction=['RPI','PGK','PGM'], 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 3PG ( 2 )
Multiplying reaction CYTBD ( 15 ) by 2.0 to avoid non-integer species O2 ( 55 )
Multiplying reaction PGK ( 54 ) by -1
Multiplying reaction PGM ( 56 ) by -1
Multiplying reaction RPI ( 65 ) by -1
```

[6]:
```
name = 'GlyPPP_abg'
reaction = []

## Glycolysis
reaction += ['PGI','PFK','FBA','TPI']
```

```
## Pentose Phosphate
reaction += ['G6PDH2R','PGL','GND','RPI','TKT2','TALA','TKT1','RPE']

ss = Extract.choose(sm,reaction=reaction)

## Create BG
ss['name'] = name
stbg.model(ss)
import GlyPPP_abg
imp.reload(GlyPPP_abg)
s = st.stoich(GlyPPP_abg.model(),quiet=quiet)
```

[6]:
```
<module 'GlyPPP_abg' from
'/home/peterg/WORK/Research/SystemsBiology/Notes/2021/Parameter/GlyPPP_abg.
 ↪py'>
```

[7]:
```
## Set up chemostats
chemostats = ['ADP','ATP','H','H2O','NADP','NADPH','CO2']
chemostats += ['G6P','G3P','R5P']
#chemostats += ['G6P','R5P']
chemostats.sort()
print(chemostats)
sc = st.statify(s,chemostats=chemostats)

sp = st.path(s,sc,pathname='PPP')
print(st.sprintp(sc))
disp.Latex(st.sprintrl(sp,chemformula=True))
```

```
['ADP', 'ATP', 'CO2', 'G3P', 'G6P', 'H', 'H2O', 'NADP', 'NADPH', 'R5P']
3 pathways
0:  + PGI + PFK + FBA + TPI
1:  + G6PDH2R + PGL + GND + RPI
2:  - 2 PGI + 2 G6PDH2R + 2 PGL + 2 GND + TKT2 + TALA + TKT1 + 2 RPE
```

[7]:

$$\mathrm{ATP + G_6P} \xrightleftharpoons{\mathrm{PPP_1}} \mathrm{ADP + 2\,G_3P + H} \tag{10}$$

$$\mathrm{G_6P + H_2O + 2\,NADP} \xrightleftharpoons{\mathrm{PPP_2}} \mathrm{CO_2 + 2\,H + 2\,NADPH + R_5P} \tag{11}$$

$$\mathrm{2\,H_2O + 4\,NADP + R_5P} \xrightleftharpoons{\mathrm{PPP_3}} \mathrm{2\,CO_2 + G_3P + 4\,H + 4\,NADPH} \tag{12}$$

[8]:
```
print(st.sprintl(sc,'K',transpose=True))
disp.Latex(st.sprintl(sc,'K',transpose=True))
```

```
\begin{align}
K^T &=
\left(\begin{array}{cccccccccccc}1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 &
0\\0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0\\-2 & 0 & 0 & 0 & 2 & 2 & 2 & 0
& 1 & 1 & 1 & 2\end{array}\right)
```

\end{align}

[8]:

$$K^T = \begin{pmatrix} 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 \\ -2 & 0 & 0 & 0 & 2 & 2 & 2 & 0 & 1 & 1 & 1 & 2 \end{pmatrix} \tag{13}$$

3.2 Extract reaction potentials Φ and deduce plausible species potentials ϕ.

Because of the energetic constaints implied by the bond graph, the reaction potentials Φ are related to the species potentials ϕ by

$$\Phi = -N^T\phi \tag{14}$$

Typically, there are more species than reactions and so N has more rows than columns. Given the reaction potentials Φ, the species potentials can be estimated using the *pseudo inverse* $N^\dagger$ of $-N^T$:

$$\hat{\phi} = N^\dagger\Phi \tag{15}$$

Notes:

- In general $\hat{\phi} \neq \phi$ but is physically plausible insofar as $-N^T\hat{\phi} = \Phi$.

[9]:
```
def getPhi(s,Phi_hyd=0.5,phi_6PGL=None,quadprog=False):
    """Extract phi for given system using
    Reaction potentials from ParRubXu16"""

    ## Reaction potentials from ParRubXu16
    PHI = phiData.Phi_ParRubXu16_Measured()

#     Phenotype = 'Mammalian'
#     Phenotype = 'Yeast'
    Phenotype = 'Ecoli'
    Phi_reac = PHI[Phenotype]

    Phi = np.zeros((len(s['reaction']),1))
    N = copy.copy(s['N'])
    N_0 = None
    N_1 = None
    Phi_0 = []
    for j,reac in enumerate(s['reaction']):
        if (reac in Phi_reac.keys()) and not np.isnan(Phi_reac[reac]):
            Phi_0.append(Phi_reac[reac])
            if N_0 is None:
                N_0 = N[:,j]
            else:
                N_0 = np.vstack((N_0,N[:,j]))
        else:
            if N_1 is None:
                N_1 = N[:,j]
            else:
                N_1 = np.vstack((N_1,N[:,j]))

    Phi_0 = np.array(Phi_0)
    #print(N_1)

    ## Compute Phi
    N_0 = N_0.T
    N_1 = N_1.T

    n_X,n_V = N_0.shape
    print(f'Extracting {n_X} values of phi from {n_V} values of Phi')


    if quadprog:
        phi = quadsolve_phi(N_0,N_1,Phi_0,Phi_min=1e-3,mu=1e-10)
    else:
        ## Compute Phi using pseudo inverse
        pinvNT =  scipy.linalg.pinv(N_0.T)
        phi = -pinvNT@Phi_0

    if phi_6PGL is not None:
        ## Reset 6PGL
        i_6PGL = s['species'].index('6PGL')
        phi[i_6PGL] = phi_6PGL
        print (f'Resetting phi_6GPL to {int(1e3*phi[i_6PGL])} mV' )

    ## Sanity check
    Phi_new = -N_0.T@phi
    err = np.linalg.norm(Phi_new-Phi_0)
    print(f'Phi error = {int(err*1000)}mV\n')

    Phi = -N.T@phi

    return Phi,phi,Phi_0,Phi_reac
```

[10]:
```
Phi_,phi_est_,Phi_0_,Phi_reac_ = getPhi(s,quadprog=False)
print('Minimum Phi = ', int(round(np.min(1e3*Phi_))), 'mV')
```

```
Extracting 19 values of phi from 10 values of Phi
Phi error = 0mV

Minimum Phi =  -3 mV
```

[11]:
```
Phi,phi_est,Phi_0,Phi_reac = getPhi(s,quadprog=True)
print('Minimum Phi = ', int(round(np.min(1e3*Phi))), 'mV')

print('\nChange in phi')
for i,spec in enumerate(s['species']):
    change = int(1e3*(phi_est[i]-phi_est_[i]))
    if not change==0:
        print(f'{i} {spec}\t {change}')

print('\nChange in Phi')
for i,reac in enumerate(s['reaction']):
    change = int(round(1e3*(Phi[i]-Phi_[i])))
    if not change == 0:
        print(f'{i} {reac}\t {change} {int(round(1e3*Phi[i]))}␣
 ↪{int(round(1e3*Phi_[i]))}')
```

```
Extracting 19 values of phi from 10 values of Phi
Phi error = 0mV

Minimum Phi =  0 mV

Change in phi
1 6PGL   1
12 H2O   1

Change in Phi
5 PGL    4 1 -3
```

3.3 Extracted reactions and reaction potentials

[12]:
```
disp.Latex(st.sprintrl(s,chemformula=True,Phi=Phi,units=['mV','kJ']␣
 ↪,showMu=showMu))
```

[12]:

$$\begin{aligned}
\mathrm{G_6P} &\xrightleftharpoons{\mathrm{PGI}} \mathrm{F_6P} && (16\ \mathrm{mV})\ [-1.59\ \mathrm{kJ\ mol^{-1}}]\\
\mathrm{ATP + F_6P} &\xrightleftharpoons{\mathrm{PFK}} \mathrm{ADP + FDP + H} && (68\ \mathrm{mV})\ [-6.64\ \mathrm{kJ\ mol^{-1}}]\\
\mathrm{FDP} &\xrightleftharpoons{\mathrm{FBA}} \mathrm{DHAP + G_3P} && (20\ \mathrm{mV})\ [-1.93\ \mathrm{kJ\ mol^{-1}}]\\
\mathrm{DHAP} &\xrightleftharpoons{\mathrm{TPI}} \mathrm{G_3P} && (7\ \mathrm{mV})\ [-0.77\ \mathrm{kJ\ mol^{-1}}]\\
\mathrm{G_6P + NADP} &\xrightleftharpoons{\mathrm{G_6PDH_2R}} \mathrm{{}_6PGL + H + NADPH} && (82\ \mathrm{mV})\ [-7.99\ \mathrm{kJ\ mol^{-1}}]\\
\mathrm{{}_6PGL + H_2O} &\xrightleftharpoons{\mathrm{PGL}} \mathrm{{}_6PGC + H} && (1\ \mathrm{mV})\ [-0.10\ \mathrm{kJ\ mol^{-1}}]\\
\mathrm{{}_6PGC + NADP} &\xrightleftharpoons{\mathrm{GND}} \mathrm{CO_2 + NADPH + RU_5PD} && (114\ \mathrm{mV})\ [-11.05\ \mathrm{kJ\ mol^{-1}}]\\
\mathrm{RU_5PD} &\xrightleftharpoons{\mathrm{RPI}} \mathrm{R_5P} && (0\ \mathrm{mV})\ [-0.00\ \mathrm{kJ\ mol^{-1}}]\\
\mathrm{E_4P + XU_5PD} &\xrightleftharpoons{\mathrm{TKT_2}} \mathrm{F_6P + G_3P} && (16\ \mathrm{mV})\ [-1.58\ \mathrm{kJ\ mol^{-1}}]\\
\mathrm{G_3P + S_7P} &\xrightleftharpoons{\mathrm{TALA}} \mathrm{E_4P + F_6P} && (54\ \mathrm{mV})\ [-5.25\ \mathrm{kJ\ mol^{-1}}]
\end{aligned}$$

$$\begin{aligned}
\mathrm{R_5P + XU_5PD} &\xrightleftharpoons{\mathrm{TKT_1}} \mathrm{G_3P + S_7P} && (4\ \mathrm{mV})\ [-0.39\ \mathrm{kJ\ mol^{-1}}]\\
\mathrm{RU_5PD} &\xrightleftharpoons{\mathrm{RPE}} \mathrm{XU_5PD} && (0\ \mathrm{mV})\ [-0.08\ \mathrm{kJ\ mol^{-1}}]
\end{aligned}$$

4 Deduce Pathway Flows

From basic stoichiometric analysis, steady-state flows can be written as:

$$f = K_p f_p \tag{16}$$

$$\text{where } K_p N^{cd} = 0 \tag{17}$$

Note that the *pathway matrix* K_p is dependent on the choice of chemostats.

Given a set of experimental flows f, an estimate $\hat{f}_p$ of f_p can be obtained from the *least-squares* formula:

$$(K_p^T K_p)\hat{f}_p = K_p^T f \tag{18}$$

Notes:

- v_p is a n_p vector containg the pathways flows
- $(K_p^T K_p)$ is a square $n_p \times n_p$ matrix where n_p is the number of pathways
- If some flows are not measured, the corresponding rows of K_p are deleted
- the reaction flows (including the missing ones) can be estimated from $\hat{f} = K_p \hat{f}_p$.
- the estimated chemostat flows are given by the non-zero elements of

$$\hat{x} = N\hat{f} \tag{19}$$

[13]:
```
def PathwayFlux(K,reaction,Reaction,flux):

    #KK = st.singleRemove(K)
    KK = K
    Kp = None
    Flux = {}
    reac_known = []
    #flux = phiData.ParRubXu16_flux()
    for i,reac in enumerate(reaction):
        if reac in flux.keys():
            reac_known.append(reac)
            fi = flux[reac]
            #Ki = np.abs(KK[i,:])
            Ki = KK[i,:]
            #print(reac,Ki)
            if Kp is None:
                Kp = Ki
                f = fi
            else:
                Kp = np.vstack((Kp,Ki))
                f = np.vstack((f,fi))
    #print(Kp)

    if Kp is not None:
        #print(f)
        f_p = np.linalg.solve(Kp.T@Kp,Kp.T@f)
        for i,Reac in enumerate(Reaction):
            Flux[Reac] = f_p[i][0]
        #print(f_p)
        f_est = Kp@f_p
        #print(Kp@f_p-f)

    error = np.linalg.norm(f_est-f)/len(f)
    print(f'Flux error = {error:.2e}')

    return Flux,f_p,f_est,f,reac_known
```

5 Reaction constants (modified mass-action) and Michaelis-Menten

The modified mass-action formula is Gawthrop et al. (2020):

$$f = \kappa \left(\exp \frac{\Phi^f}{\alpha V_N} - \exp \frac{\Phi^r}{\alpha V_N} \right) \tag{20}$$

Thus an estimate for κ can be computed as:

$$\hat{\kappa} = \frac{\hat{f}}{f_0} \tag{21}$$

$$\text{where } f_0 = \left(\exp \frac{\Phi^f}{\alpha V_N} - \exp \frac{\Phi^r}{\alpha V_N} \right) \tag{22}$$

```
[14]: def reactionConstant(s,phi_est,f_est,alpha=1,K_E=100,K_C=1,rho=0.9):

    V_N = st.V_N()

    ## Extract stoichiometry
    N = s['N']
    Nf = s['Nf']
    Nr = s['Nr']
    reaction = s['reaction']

    ## Compute Phis from estimated phi
    Phi_ = -N.T@phi_est
    Phi_f = Nf.T@phi_est
    Phi_r = Nr.T@phi_est

    ## Compute normalised flow rates

    f_plus  = np.exp(Phi_f/(alpha*V_N))
    f_minus = np.exp(Phi_r/(alpha*V_N))
    f0 = f_plus - f_minus


    parameter = {}
    MMparameter = {}
    for i,react in enumerate(reaction):
        MMpar = {}
        kap = f_est[i][0]/f0[i]
        parameter[f'kappa_{react}'] = kap
        #print(f'{react}: \tPhi = {int(Phi_[i]*1000)}mV, \tf_est =␣
↪{f_est[i][0]:.2e}, \tkappa = {kap:.2}')

        ## MM version
        X_data = np.array([1,f_est[i][0],-f_est[i][0]/f0[i]])
        Y_data = f_est[i][0]*f_plus[i]/f0[i]
#         print(X_data)
#         print(Y_data)

        XTX = np.outer(X_data,X_data)
        XTy = X_data*Y_data

#         print('XTX:', XTX)
#         print('XTy:', XTy)

        ## Pseuso inverse eapproach
#         theta = np.linalg.pinv(XTX)@XTy
#         print(theta)

        ## QP approach
#         f_max_est = 10
        rho_est = rho
        k_v_est = K_C/K_E

        G = -np.eye(3)
        h = -0*np.ones(3)
        A_eq = np.array([[0,1,0],[0,0,1]])
        b_eq = np.array([rho_est,k_v_est])

#         A_eq = np.array([[1,0,0],[0,1,0]])
#         b_eq = np.array([f_max_est,rho_est])


        theta = quadprog_solve_qp(XTX+1e-10*np.eye(3),-XTy,G,h,A_eq,b_eq)
        f_max = theta[0]
        rho = theta[1]
        k_v = theta[2]
        kappa = f_max/k_v

        MMpar['f_max'] = theta[0]
        MMpar['rho'] = theta[1]
        MMpar['k_v'] = theta[2]
#         print(f'{react}: kappa={kappa:6.2f} f_max={f_max:.2} rho={rho:0.2f}␣
↪k_v={k_v:6.2f}')

        MMparameter[react] = MMpar

    return parameter,MMparameter
```

[15]:
```
## Convert to BG parameters
def MMtoBG(MMpar,K_E=100):
    kappa = np.zeros(2)
    K_CE = np.zeros(2)
    rho = MMpar['rho']
    K_C = K_E*MMpar['k_v']
#     print(MMpar)
    kappa_bar = MMpar['f_max']/K_C
    kappa[0] = kappa_bar/rho
    kappa[1] = kappa_bar/(1-rho)

    K_CE[0] = K_C
    K_CE[1] = K_E
#     print(kappa_1,kappa_2,K_E)

    return kappa,K_CE

```

5.1 Normalise data

[16]:
```
imp.reload(phiData)
## Extract experimetal data
Concentration = phiData.ParRubXu16_conc() # M
concentration = Concentration['Ecoli']

Flux = phiData.ParRubXu16_flux() # mM/min
flux = Flux['Ecoli']

c_0 = concentration['G6P']
f_0 = flux['PGI']/60
t_0 = (1e3*c_0)/f_0

print(f'c_0 = {c_0*1000} mM, f_0 = {f_0} mM/sec, t_0 = {t_0} sec')
```

[16]:
```
<module 'phiData' from
'/home/peterg/WORK/Research/SystemsBiology/lib/python/phiData.py'>
```

```
c_0 = 7.88 mM, f_0 = 0.9916666666666667 mM/sec, t_0 = 7.946218487394957 sec
```

5.2 Show computed reaction flows

[17]:
```
K = sc['K']
n_path = K.shape[1]
Reaction = []
for i in range(n_path):
    Reaction += [f'PPP{i+1}']

    print(Reaction)

for reac in flux.keys():
    flux[reac] *= 1/f_0
```

```
fluxp,f_p,f_est,f,reaction = PathwayFlux(sc['K'],s['reaction'],Reaction,flux)

## Assumed values:
K_E = 10
K_C = 1
rho = 0.2


## Reaction constants
f_est = sc['K']@f_p
parameter,MMparameter =␣
 ↪reactionConstant(s,phi_est,f_est,K_E=K_E,K_C=K_C,rho=rho)
K_C=K_C
#f_est = sc['K']@f_p
j=0

print('\n\n%% LaTeX table')
print('\\hline')
print('Reaction &\t $\Phi$~mV &\t $\hat{\Phi}$~mV &\t $f$ & $\\hat{f}$ \
& $\\hat{\\kappa}$ & $\\hat{\\kappa_1}$ & $\\hat{\\kappa_2}$\\\\')
print('\\hline')
for i,reac in enumerate(s['reaction']):

    ## BG MM equivalent
    MMpar = MMparameter[reac]
    kappa_MM,K_CE = MMtoBG(MMpar,K_E=100.0)

    if reac in flux.keys():
        ff = f'{f[j][0]:0.2f}'
        j += 1
    else:
        ff = '--'
    if reac in Phi_reac.keys():
        PP = f'{1e3*Phi_reac[reac]:.2f}'
    else:
        PP = '--'
    kappa = 'kappa_'+reac
    print(
        f'{reac} &\t {PP} &\t {1e3*Phi[i]:.2f} &\t {ff} & {f_est[i][0]:0.2f} \
        & {parameter[kappa]:.2f} & {kappa_MM[0]:.2f} & {kappa_MM[1]:.2f} \\\\'
    )
print('\\hline')
```

```
['PPP1']
['PPP1', 'PPP2']
['PPP1', 'PPP2', 'PPP3']
Flux error = 1.86e-01


%% LaTeX table
```

```
\hline
Reaction &      $\Phi$~mV &     $\hat{\Phi}$~mV &       $f$ & $\hat{f}$ &
$\hat{\kappa}$ & $\hat{\kappa_1}$ & $\hat{\kappa_2}$\\
\hline
PGI &    16.48 &        16.48 &        60.00 & 59.52        & 154.39 & 66.44
& 16.61 \\
PFK &    68.82 &        68.82 &        62.62 & 63.12        & 54.85 & 30.59 &
7.65 \\
FBA &    20.00 &        20.00 &        63.43 & 63.12        & 160.08 & 61.59
& 15.40 \\
TPI &    7.98 &  7.98 &  62.82 & 63.12        & 353.93 & 133.64 & 33.41 \\
G6PDH2R &       -- &    82.84 &        -- & 11.58        & 4.67 & 5.14 & 1.28
\\
PGL &    -- &    1.00 &  -- & 11.58        & 291.64 & 171.06 & 42.77 \\
GND &    114.53 &       114.53 &       11.70 & 11.58        & 1.27 & 4.78 &
1.19 \\
RPI &    0.04 &  0.04 &  7.87 & 7.98        & 4206.98 & 2785.35 & 696.34 \\
TKT2 &   16.38 &        16.38 &        0.91 & 1.80        & 9.17 & 2.24 &
0.56 \\
TALA &   54.41 &        54.41 &        -- & 1.80        & 1.66 & 0.94 & 0.23
\\
TKT1 &   4.04 &  4.04 &  2.92 & 1.80        & 8.82 & 6.66 & 1.67 \\
RPE &    0.83 &  0.83 &  3.83 & 3.59        & 96.07 & 63.27 & 15.82 \\
\hline
```

5.3 Show computed chemostat flows

[18]:
```python
dx_est = s['N']@f_est

print('\n\n%% LaTeX table')
print('\\hline')
print('Chemostat &\t flow \\\\')
print('\\hline')
for i,spec in enumerate(s['species']):
    if spec in chemostats:
        print(f'{spec} &\t {dx_est[i][0]:0.2f} \\\\')
print('\\hline')
```

```
%% LaTeX table
\hline
Chemostat &      flow \\
\hline
ADP &    63.12 \\
ATP &    -63.12 \\
CO2 &    11.58 \\
G3P &    128.03 \\
G6P &    -71.10 \\
H &      86.27 \\
H2O &    -11.58 \\
NADP &   -23.16 \\
NADPH &  23.16 \\
R5P &    6.19 \\
\hline
```

5.4 Show pathway flows

```
[19]: print('\n\n%% LaTeX table')
      print('\\hline')
      print('Pathway &\t $\hat{f}_p$ \\\\')
      print('\\hline')
      for reac in fluxp.keys():
          print(f'{reac} &\t {fluxp[reac]:0.2f} \\\\')
      print('\\hline')
```

```
%% LaTeX table
\hline
Pathway &        $\hat{f}_p$ \\
\hline
PPP1 &   63.12 \\
PPP2 &   7.98 \\
PPP3 &   1.80 \\
\hline
```

6 Species constants

$$K = \frac{\exp \phi}{x^\circ} = \frac{\exp \phi}{V c^\circ} \tag{23}$$

```
[20]: #imp.reload(phiData)

      print('\n\n%% LaTeX table')
      print('\\hline')
      print('Species &\t $\\hat{\\phi}~mV$ & $\\frac{c}{c_0}$ & $\\hat{K}$ \\\\')
      print('\\hline')


      #concentration['H'] = 1e-7

      ## Data in mM
      scale = 1e3
      K_spec = np.ones(s['n_X'])
      conc = np.ones(s['n_X'])
      c_G6P = concentration['G6P']
      #print('c_G6P',c_G6P)
      for i,spec in enumerate(s['species']):
          if spec in concentration.keys():
              conc[i] = concentration[spec]/c_G6P
              K_spec[i] = np.exp(phi_est[i]/st.V_N())/conc[i]
```

```
        print(f'{spec} & {int(round(1e3*phi_est[i]))} &  \t{conc[i]:.4f} &␣
 ↪{K_spec[i]:.4f} \\\\')
    else:
        K_spec[i] = np.exp(phi_est[i]/st.V_N())
#          print(f'{spec} &{phi_est[i]:.2} & -- & --\\\\')

print('\\hline')

#print(conc)
print(s['species'])
```

```
%% LaTeX table
\hline
Species &        $\hat{\phi}~mV$ & $\frac{c}{c_0}$ & $\hat{K}$ \\
\hline
6PGC & 29 &     0.4784 & 6.2335 \\
ADP & -27 &     0.0704 & 5.1546 \\
ATP & 27 &      1.2221 & 2.2539 \\
CO2 & -30 &     0.0095 & 33.7942 \\
DHAP & -10 &    0.3883 & 1.7790 \\
E4P & -27 &     0.0062 & 57.9353 \\
F6P & -21 &     0.3198 & 1.4140 \\
FDP & -8 &      1.9289 & 0.3880 \\
G3P & -18 &     0.0344 & 14.9020 \\
G6P & -5 &      1.0000 & 0.8377 \\
NADP & 30 &     0.0003 & 11747.0633 \\
NADPH & -30 &   0.0154 & 21.0027 \\
R5P & 5 &       0.0999 & 12.2419 \\
RU5PD & 5 &     0.0142 & 86.1551 \\
S7P & 24 &      0.1119 & 21.7513 \\
XU5PD & 5 &     0.0230 & 51.6829 \\
\hline
['6PGC', '6PGL', 'ADP', 'ATP', 'CO2', 'DHAP', 'E4P', 'F6P', 'FDP', 'G3P', 'G6P',
'H', 'H2O', 'NADP', 'NADPH', 'R5P', 'RU5PD', 'S7P', 'XU5PD']
```

7 Simulation

7.1 Set up parameters

- Reaction constants already set

[21]:
```
for i,spec in enumerate(s['species']):
    #K_spec = np.exp(phi_est[i]/st.V_N())
    parameter['K_'+spec] = K_spec[i]
```

[ ]:
```
```

7.2 Set up chemostats and flowstats

[22]:
```
def setPath(s,path='R5P'):

    print('\n Path =', path)

    if path == 'R5P':
        chemostats = ['ADP', 'ATP', 'CO2', 'G6P', 'H', 'H2O', 'NADP',␣
↪'NADPH', 'R5P']
        flowstats =['G6PDH2R']
        dX_G6P = 5
    elif path == 'NADPH':
        chemostats = ['ADP', 'ATP', 'CO2', 'G6P', 'H', 'H2O', 'NADP', 'NADPH']
        flowstats = []
        dX_G6P = 1
    elif path == 'both':
        chemostats = ['ADP', 'ATP', 'CO2', 'G6P', 'H', 'H2O', 'NADP',␣
↪'NADPH', 'R5P']
        flowstats =  ['PGI', 'TKT2']
        dX_G6P = 1
    elif path == 'all':
        chemostats = ['ADP', 'ATP', 'CO2', 'G6P', 'H', 'H2O', 'NADP',␣
↪'NADPH', 'R5P','G3P']
        flowstats = []
        dX_G6P = 10

    sc = st.statify(s,chemostats=chemostats)
    sf = st.statify(s,flowstats=flowstats)

    return sc,sf,dX_G6P
```

7.3 Time unit

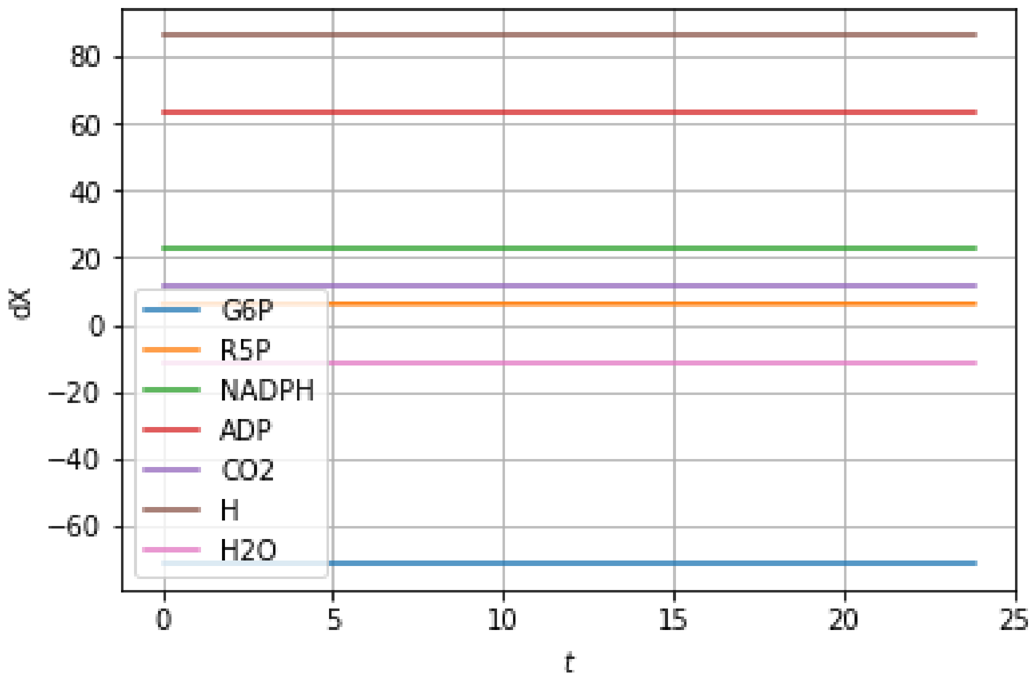
[23]:
```
##t_0 = ((1000*c_G6P)/flux_PGI)*100
print(f"Time unit: {t_0:4.2f} sec")
```

```
Time unit: 7.95 sec
```

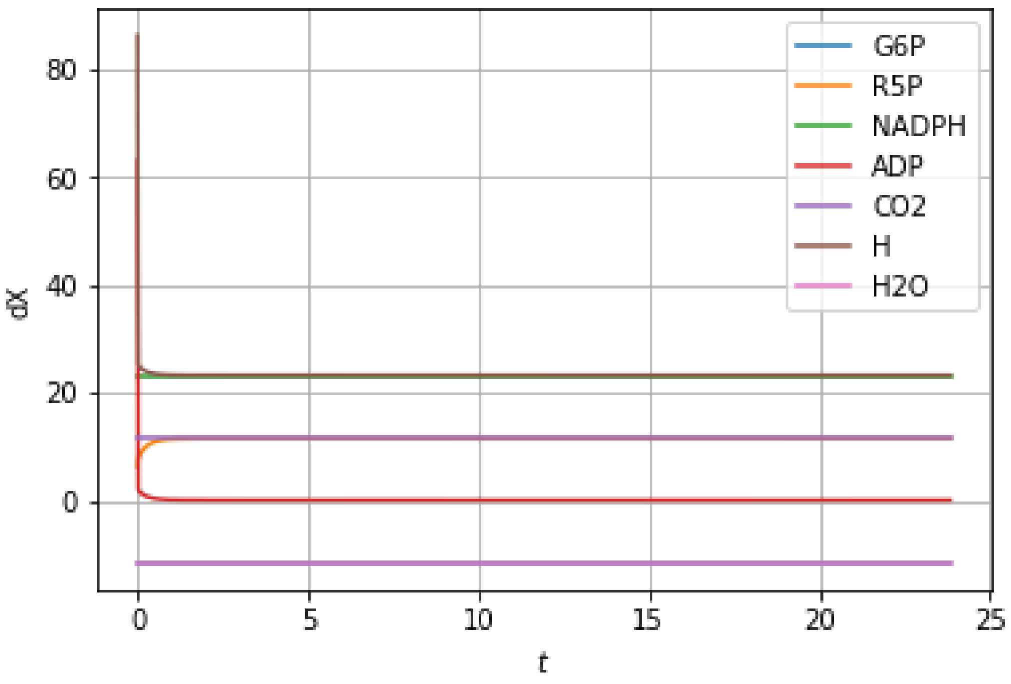
7.4 Simulation

[24]:
```
approximateFlowstats = True

Spec = ['G6P','R5P','NADPH','ADP','CO2','H','H2O']
paths = ['all','both','R5P','NADPH']
#paths = ['R5P']
RATIO = {}
for path in paths:
    Ratio = {}
    normalisedRatio = {}

    ## Set up pathway]
    spec = sc['species']
    sc,sf,dX_G6P_0 = setPath(s,path=path)

    ## Set up parameters
    par = copy.copy(parameter)
    if approximateFlowstats:
        small = 1e-3
        par = copy.copy(parameter)
        for fs in sf['flowstats']:
            par['kappa_'+fs] = small
        sf = None

    ## Simulate
    t = np.linspace(0,3*t_0,1000)

#     ## Find steady-state with no flowstats
#     dat_ss = st.sim(s,sc=sc,sf=sf,t=t,parameter=parameter,X0=conc)
#     X_ss = dat_ss['X'][-1,:]

    dat = st.sim(s,sc=sc,sf=sf,t=t,parameter=par,X0=conc)
    #st.plot(s,dat,species=[])
    st.plot(s,dat,reaction=[],species=Spec,dX=True)

    ## Extract some external flows
    DX = dat['dX']
    dX = {}
    for Sp in Spec:
        dX[Sp] = DX[:,spec.index(Sp)]
        Ratio[Sp] = -dX[Sp]/dX['G6P']
        normalisedRatio[Sp] =  -dX_G6P_0*dX[Sp]/dX['G6P']

    RATIO[path] = normalisedRatio

    ## Print steady-state values
    for Sp in Spec:
        ratio = Ratio[Sp][-1]
        print(f'{Sp}:\t{dX[Sp][0]:3.1f} \t{dX[Sp][-1]:3.
 ↪1f}\t{(dX_G6P_0*ratio):3.1f}\t{100*ratio:3.1f}%')
```

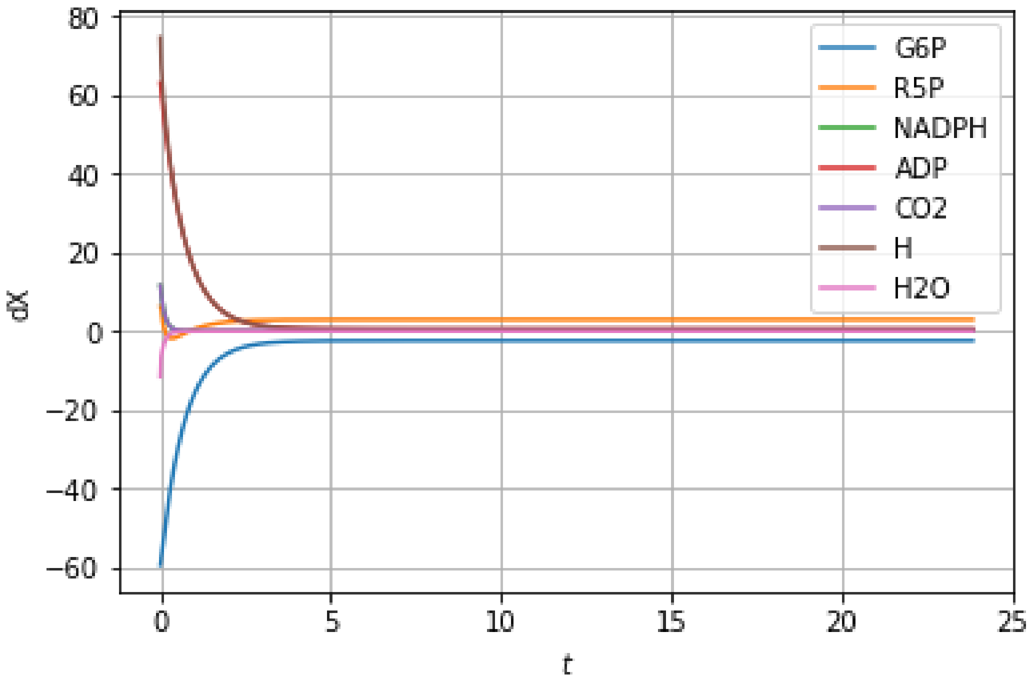
```
Path = all
```



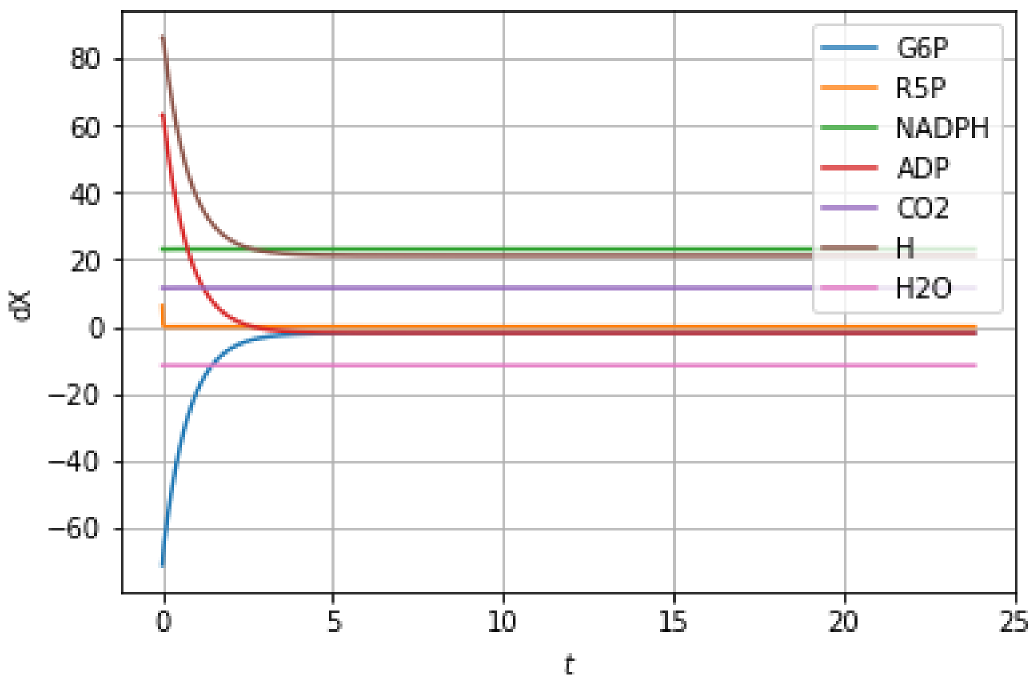
```
G6P:    -71.1   -71.1   -10.0   -100.0%
R5P:    6.2     6.2     0.9     8.7%
NADPH:  23.2    23.2    3.3     32.6%
ADP:    63.1    63.1    8.9     88.8%
CO2:    11.6    11.6    1.6     16.3%
H:      86.3    86.3    12.1    121.3%
H2O:    -11.6   -11.6   -1.6    -16.3%

 Path = both
```



```
G6P:     -11.6    -11.6    -1.0     -100.0%
R5P:     6.2      11.5     1.0      99.4%
NADPH:   23.2     23.2     2.0      200.0%
ADP:     63.1     0.0      0.0      0.4%
CO2:     11.6     11.6     1.0      100.0%
H:       86.3     23.2     2.0      200.4%
H2O:     -11.6    -11.6    -1.0     -100.0%

 Path = R5P
```



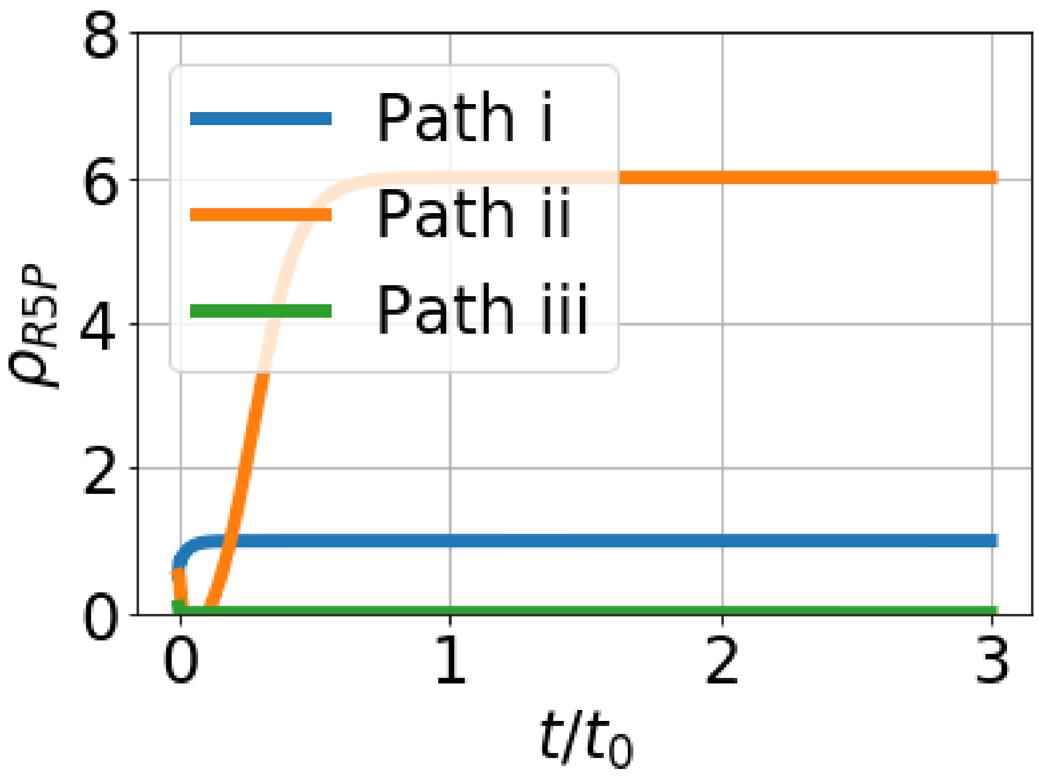
```
G6P:    -59.5   -2.4    -5.0    -100.0%
R5P:    6.2     2.9     6.0     120.0%
NADPH:  11.6    0.0     0.0     0.2%
ADP:    63.1    0.5     1.0     20.0%
CO2:    11.6    0.0     0.0     0.1%
H:      74.7    0.5     1.0     20.2%
H2O:    -11.6   -0.0    -0.0    -0.1%

 Path = NADPH
```



```
G6P:     -71.1    -1.9     -1.0     -100.0%
R5P:     6.2      0.0      0.0      0.0%
NADPH:   23.2     23.1     12.0     1200.0%
ADP:     63.1     -1.9     -1.0     -100.0%
CO2:     11.6     11.6     6.0      600.0%
H:       86.3     21.2     11.0     1100.0%
H2O:     -11.6    -11.6    -6.0     -600.0%
```

[25]:
```
## Plot ratios
name = ['i','ii','iii']
for sp in ['R5P','NADPH']:
    BigFont = 24
    plt.rcParams.update({'font.size': BigFont})
    for i,path in enumerate(['both','R5P','NADPH']):
        Ratio = RATIO[path]
        label = f'Path {name[i]}'
        plt.plot(t/t_0,Ratio[sp],label=label,linewidth=5)
    if sp=='R5P':
        ylim = 8
    else:
        ylim=15
    plt.ylim((0,ylim))
    ylabel = r'$\rho_{'+sp+'}$'
    plt.ylabel(ylabel)
    plt.xlabel('$t/t_0$')
    plt.legend()
    plt.grid()
    if SaveFig:
        plt.savefig(f'Figs/{sp}.pdf',bbox_inches='tight')

    plt.show()
```

```
[25]: [<matplotlib.lines.Line2D at 0x7fc3108331c0>]

[25]: [<matplotlib.lines.Line2D at 0x7fc3108336d0>]

[25]: [<matplotlib.lines.Line2D at 0x7fc310833c10>]

[25]: (0, 8)

[25]: Text(0,0.5,'$\\rho_{R5P}$')

[25]: Text(0.5,0,'$t/t_0$')

[25]: <matplotlib.legend.Legend at 0x7fc310833160>
```

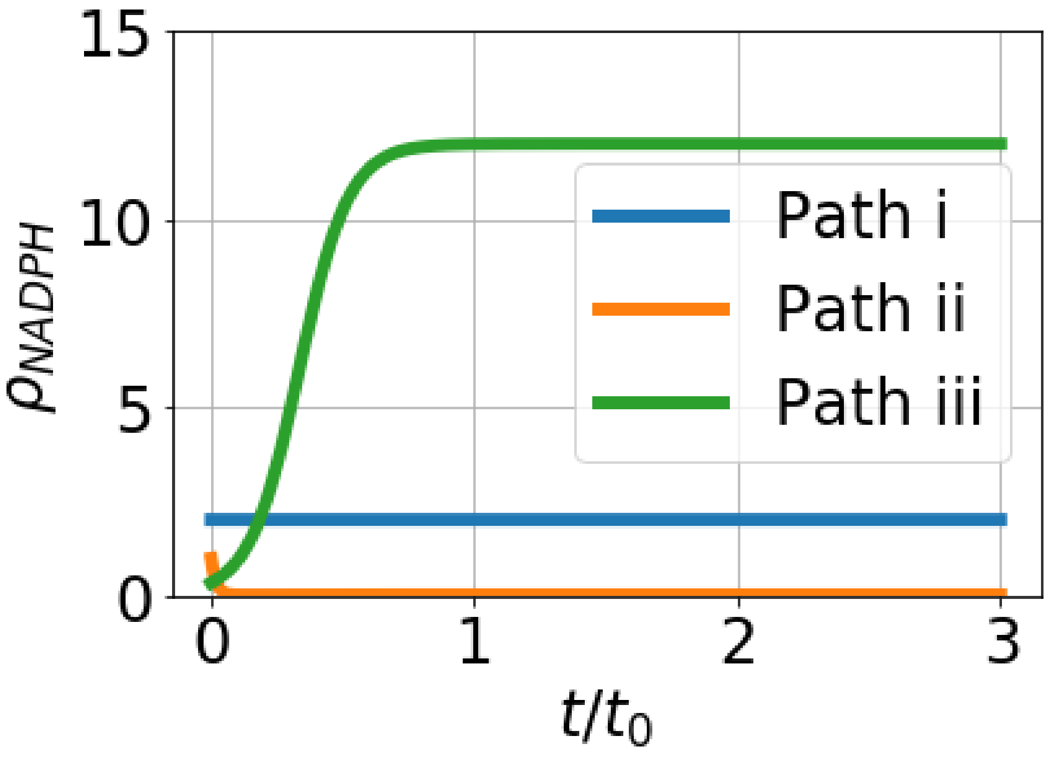


```
[25]: [<matplotlib.lines.Line2D at 0x7fc31080ed30>]

[25]: [<matplotlib.lines.Line2D at 0x7fc316d63ee0>]

[25]: [<matplotlib.lines.Line2D at 0x7fc310814220>]

[25]: (0, 15)
```

```
[25]: Text(0,0.5,'$\\rho_{NADPH}$')
```

```
[25]: Text(0.5,0,'$t/t_0$')
```

```
[25]: <matplotlib.legend.Legend at 0x7fc31080eca0>
```



```
[26]: X = np.array([1,2,3])
      print(X)
      print(X.T)
      print(np.outer(X,X))
      print(np.linalg.pinv(np.outer(X,X)))
      print(np.linalg.pinv(np.outer(X,X))@X.T)
      print(X@X.T)
```

```
[1 2 3]
[1 2 3]
[[1 2 3]
 [2 4 6]
 [3 6 9]]
[[0.00510204 0.01020408 0.01530612]
 [0.01020408 0.02040816 0.03061224]
 [0.01530612 0.03061224 0.04591837]]
[0.07142857 0.14285714 0.21428571]
14
```

8 Michaelis-Menten formulation

8.1 Show results

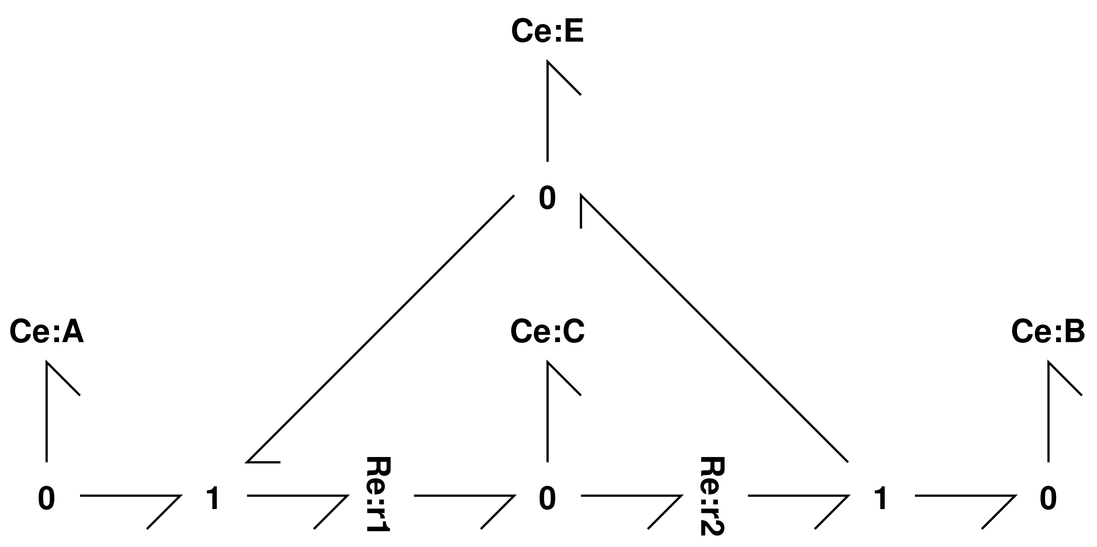
[27]:
```
for reac in reaction:
    MMpar = MMparameter[reac]
    f_max = MMpar['f_max']
    rho = MMpar['rho']
    k_v = MMpar['k_v']
    print(f'Reaction {reac}:')
    print(f'f_max = {f_max:6.2f}; rho = {rho:1.2f}; k_v = {k_v:4.2f}')
    kappa,K_CE = MMtoBG(MMpar,K_E=K_E)
    print(f'kappa_1 = {kappa[0]:3.1f}; kappa_2 = {kappa[1]:3.1f}; K_C =␣
 ↪{K_CE[0]:3.1f}; K_E = {K_CE[1]:3.1f}')
```

```
Reaction PGI:
f_max = 132.87; rho = 0.20; k_v = 0.10
kappa_1 = 664.4; kappa_2 = 166.1; K_C = 1.0; K_E = 10.0
Reaction PFK:
f_max =  61.18; rho = 0.20; k_v = 0.10
kappa_1 = 305.9; kappa_2 = 76.5; K_C = 1.0; K_E = 10.0
Reaction FBA:
f_max = 123.18; rho = 0.20; k_v = 0.10
kappa_1 = 615.9; kappa_2 = 154.0; K_C = 1.0; K_E = 10.0
Reaction TPI:
f_max = 267.28; rho = 0.20; k_v = 0.10
kappa_1 = 1336.4; kappa_2 = 334.1; K_C = 1.0; K_E = 10.0
Reaction GND:
f_max =   9.55; rho = 0.20; k_v = 0.10
kappa_1 = 47.8; kappa_2 = 11.9; K_C = 1.0; K_E = 10.0
Reaction RPI:
f_max = 5570.71; rho = 0.20; k_v = 0.10
kappa_1 = 27853.5; kappa_2 = 6963.4; K_C = 1.0; K_E = 10.0
Reaction TKT2:
f_max =   4.48; rho = 0.20; k_v = 0.10
kappa_1 = 22.4; kappa_2 = 5.6; K_C = 1.0; K_E = 10.0
Reaction TKT1:
f_max =  13.32; rho = 0.20; k_v = 0.10
kappa_1 = 66.6; kappa_2 = 16.7; K_C = 1.0; K_E = 10.0
Reaction RPE:
f_max = 126.53; rho = 0.20; k_v = 0.10
kappa_1 = 632.7; kappa_2 = 158.2; K_C = 1.0; K_E = 10.0
```

8.2 Bond graph model of Enzyme Catalysed Reaction (RE)

[28]:
```
sbg.model('RE_abg.svg')
import RE_abg as RE
disp.SVG('RE_abg.svg')
```

[28]:



[29]:
```
sRE = st.stoich(RE.model(),quiet=quiet)
disp.Latex(st.sprintrl(sRE,chemformula=True))
```

[29]:

$$A + E \xrightleftharpoons{r_1} C \tag{24}$$
$$C \xrightleftharpoons{r_2} B + E \tag{25}$$

[30]:
```
stbg.model(s,filename='PPP_abg')
import PPP_abg
PPP = PPP_abg.model()
disp.Latex(st.sprintrl(s,chemformula=True))
```

[30]:

$$G_6P \xrightleftharpoons{PGI} F_6P \tag{26}$$
$$ATP + F_6P \xrightleftharpoons{PFK} ADP + FDP + H \tag{27}$$
$$FDP \xrightleftharpoons{FBA} DHAP + G_3P \tag{28}$$
$$DHAP \xrightleftharpoons{TPI} G_3P \tag{29}$$
$$G_6P + NADP \xrightleftharpoons{G_6PDH_2R} {}_6PGL + H + NADPH \tag{30}$$
$$_6PGL + H_2O \xrightleftharpoons{PGL} {}_6PGC + H \tag{31}$$
$$_6PGC + NADP \xrightleftharpoons{GND} CO_2 + NADPH + RU_5PD \tag{32}$$
$$RU_5PD \xrightleftharpoons{RPI} R_5P \tag{33}$$
$$E_4P + XU_5PD \xrightleftharpoons{TKT_2} F_6P + G_3P \tag{34}$$
$$G_3P + S_7P \xrightleftharpoons{TALA} E_4P + F_6P \tag{35}$$

$$\mathrm{R_5P + XU_5PD} \xrightleftharpoons{\mathrm{TKT_1}} \mathrm{G_3P + S_7P} \quad (36)$$

$$\mathrm{RU_5PD} \xrightleftharpoons{\mathrm{RPE}} \mathrm{XU_5PD} \quad (37)$$

8.3 Replace Re components by RE

```
[31]: imp.reload(mbg)
      mbg.ReRE(PPP,quiet=quiet)
```

```
[31]: <module 'modularBondGraph' from
      '/home/peterg/WORK/Research/SystemsBiology/lib/python/modularBondGraph.py'>
```

```
[32]: sPPPRE = st.stoich(PPP,quiet=quiet)
```

```
[ ]:
```

```
[33]: disp.Latex(st.sprintrl(sPPPRE,chemformula=True))
```

[33]:

$$\mathrm{G_6P + PGIase} \xrightleftharpoons{\mathrm{PGI_1}} \mathrm{PGIcmp} \quad (38)$$

$$\mathrm{PGIcmp} \xrightleftharpoons{\mathrm{PGI_2}} \mathrm{F_6P + PGIase} \quad (39)$$

$$\mathrm{ATP + F_6P + PFKase} \xrightleftharpoons{\mathrm{PFK_1}} \mathrm{PFKcmp} \quad (40)$$

$$\mathrm{PFKcmp} \xrightleftharpoons{\mathrm{PFK_2}} \mathrm{ADP + FDP + H + PFKase} \quad (41)$$

$$\mathrm{FDP + FBAase} \xrightleftharpoons{\mathrm{FBA_1}} \mathrm{FBAcmp} \quad (42)$$

$$\mathrm{FBAcmp} \xrightleftharpoons{\mathrm{FBA_2}} \mathrm{DHAP + G_3P + FBAase} \quad (43)$$

$$\mathrm{DHAP + TPIase} \xrightleftharpoons{\mathrm{TPI_1}} \mathrm{TPIcmp} \quad (44)$$

$$\mathrm{TPIcmp} \xrightleftharpoons{\mathrm{TPI_2}} \mathrm{G_3P + TPIase} \quad (45)$$

$$\mathrm{G_6P + NADP + G_6PDH_2Rase} \xrightleftharpoons{\mathrm{G_6PDH_2R_1}} \mathrm{G_6PDH_2Rcmp} \quad (46)$$

$$\mathrm{G_6PDH_2Rcmp} \xrightleftharpoons{\mathrm{G_6PDH_2R_2}} \mathrm{{}_6PGL + H + NADPH + G_6PDH_2Rase} \quad (47)$$

$$\mathtt{{}_6PGL + H_2O + PGLase} \xrightleftharpoons{\mathtt{PGL_1}} \mathtt{PGLcmp} \tag{48}$$
$$\mathtt{PGLcmp} \xrightleftharpoons{\mathtt{PGL_2}} \mathtt{{}_6PGC + H + PGLase} \tag{49}$$
$$\mathtt{{}_6PGC + NADP + GNDase} \xrightleftharpoons{\mathtt{GND_1}} \mathtt{GNDcmp} \tag{50}$$
$$\mathtt{GNDcmp} \xrightleftharpoons{\mathtt{GND_2}} \mathtt{CO_2 + NADPH + RU_5PD + GNDase} \tag{51}$$
$$\mathtt{RU_5PD + RPIase} \xrightleftharpoons{\mathtt{RPI_1}} \mathtt{RPIcmp} \tag{52}$$
$$\mathtt{RPIcmp} \xrightleftharpoons{\mathtt{RPI_2}} \mathtt{R_5P + RPIase} \tag{53}$$
$$\mathtt{E_4P + XU_5PD + TKT_2ase} \xrightleftharpoons{\mathtt{TKT_{21}}} \mathtt{TKT_2cmp} \tag{54}$$
$$\mathtt{TKT_2cmp} \xrightleftharpoons{\mathtt{TKT_{22}}} \mathtt{F_6P + G_3P + TKT_2ase} \tag{55}$$
$$\mathtt{G_3P + S_7P + TALAase} \xrightleftharpoons{\mathtt{TALA_1}} \mathtt{TALAcmp} \tag{56}$$
$$\mathtt{TALAcmp} \xrightleftharpoons{\mathtt{TALA_2}} \mathtt{E_4P + F_6P + TALAase} \tag{57}$$

$$\mathtt{R_5P + XU_5PD + TKT_1ase} \xrightleftharpoons{\mathtt{TKT_{11}}} \mathtt{TKT_1cmp} \tag{58}$$
$$\mathtt{TKT_1cmp} \xrightleftharpoons{\mathtt{TKT_{12}}} \mathtt{G_3P + S_7P + TKT_1ase} \tag{59}$$
$$\mathtt{RU_5PD + RPEase} \xrightleftharpoons{\mathtt{RPE_1}} \mathtt{RPEcmp} \tag{60}$$
$$\mathtt{RPEcmp} \xrightleftharpoons{\mathtt{RPE_2}} \mathtt{XU_5PD + RPEase} \tag{61}$$

[34]:
```
print(chemostats)
```

```
['ADP', 'ATP', 'CO2', 'G3P', 'G6P', 'H', 'H2O', 'NADP', 'NADPH', 'R5P']
```

[35]:
```
scPPPRE = st.statify(sPPPRE,chemostats=chemostats)
```

8.4 Set up parameters

[36]:
```
parameter = {}
for i,spec in enumerate(s['species']):
    parameter['K_'+spec] = K_spec[i]

for reac in reaction:
    MMpar = MMparameter[reac]
    kappa,K_CE = MMtoBG(MMpar,K_E=100.0)
    for i in ['1','2']:
        Kappa = f'kappa_{reac}{i}'
        parameter[Kappa] = kappa[int(i)-1]
    for i,spec in enumerate(['cmp','ase']):
        K = f'K_{reac}{spec}'
        parameter[K] = K_CE[i]

# print(parameter)
```

[37]:
```
## Initial conds
n_X = sPPPRE['n_X']
X0 = 0.5*np.ones(n_X)
for i,spec in enumerate(sPPPRE['species']):
    if spec in s['species']:
        X0[i] = conc[s['species'].index(spec)]
```

8.5 Simulate

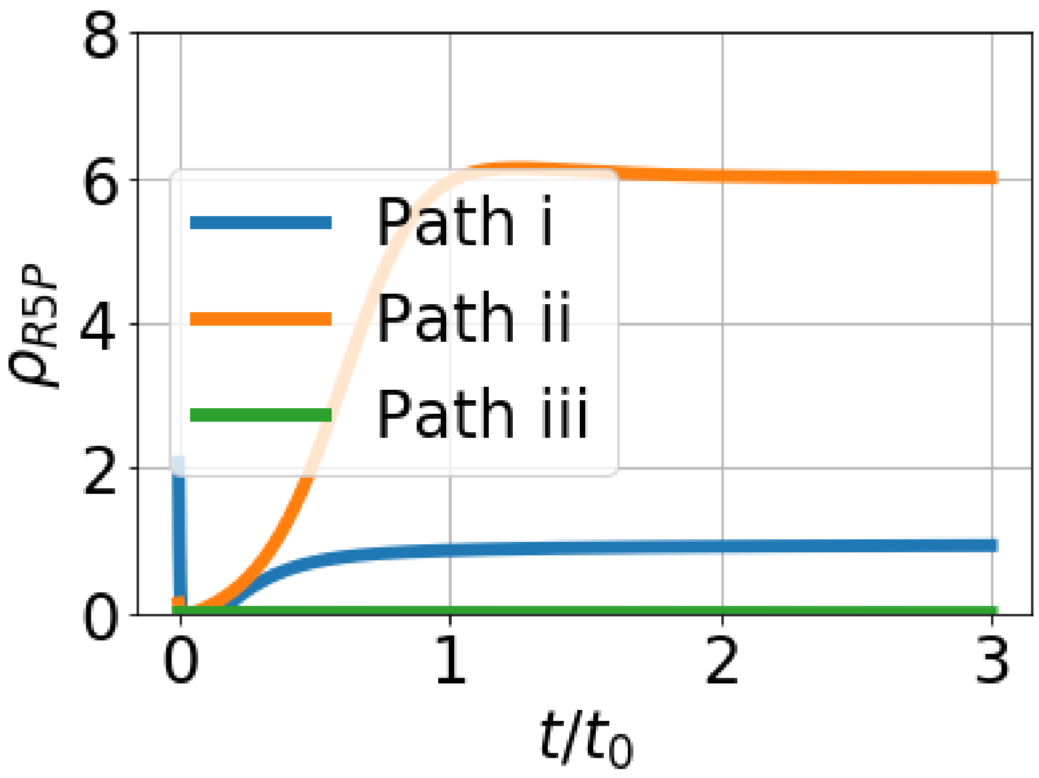
[38]:
```
approximateFlowstats = True

Spec = ['G6P','R5P','NADPH','ADP','CO2','H','H2O']
paths = ['all','both','R5P','NADPH']
#paths = ['R5P']
RATIO = {}
for path in paths:
    Ratio = {}
    normalisedRatio = {}

    ## Set up pathway]
    spec = sPPPRE['species']
    sc,sf,dX_G6P_0 = setPath(sPPPRE,path=path)

    ## Set up parameters
    par = copy.copy(parameter)
    if approximateFlowstats:
        small = 1e-3
        par = copy.copy(parameter)
        for fs in sf['flowstats']:
            par['kappa_'+fs+'1'] = small
            par['kappa_'+fs+'2'] = small
        sf = None

    ## Find the initial condion X_ss after the initial transient due to E/C
    t_ss = np.linspace(0,t_0/100,100)
    dat_ss = st.sim(sPPPRE,sc=sc,t=t_ss,parameter=parameter,X0=X0)
    X_ss = dat_ss['X'][-1,:]

    ## Simulate from after transient
    dat = st.sim(sPPPRE,sc=sc,t=t,parameter=par,X0=X_ss)
#     st.plot(s,dat,reaction=[],species=Spec,dX=True)

    ## Extract some external flows
    DX = dat['dX']
    dX = {}
    for Sp in Spec:
        dX[Sp] = DX[:,spec.index(Sp)]
        Ratio[Sp] = -dX[Sp]/dX['G6P']
        normalisedRatio[Sp] =  -dX_G6P_0*dX[Sp]/dX['G6P']

    RATIO[path] = normalisedRatio
    ## Print steady-state values
    for Sp in Spec:
        ratio = Ratio[Sp][-1]
        print(f'{Sp}:\t{dX[Sp][0]:3.1f} \t{dX[Sp][-1]:3.
↪1f}\t{(dX_G6P_0*ratio):3.1f}\t{100*ratio:3.1f}%')
```

```
 Path = all
G6P:    -63.8   -62.5   -10.0   -100.0%
R5P:    0.8     -0.7    -0.1    -1.0%
NADPH:  2.3     1.0     0.2     1.6%
ADP:    62.8    62.8    10.0    100.4%
CO2:    1.8     0.5     0.1     0.8%
H:      63.7    63.7    10.2    102.0%
H2O:    -0.1    -0.5    -0.1    -0.8%

 Path = both
Flowstat PGI is not a model reaction
Flowstat TKT2 is not a model reaction
G6P:    -0.7    -0.5    -1.0    -100.0%
R5P:    1.5     0.5     0.9     92.9%
NADPH:  2.3     1.0     2.0     197.1%
ADP:    53.3    0.0     0.0     4.7%
CO2:    1.8     0.5     1.0     98.2%
H:      54.2    1.0     2.0     201.8%
H2O:    -0.1    -0.5    -1.0    -98.3%

 Path = R5P
Flowstat G6PDH2R is not a model reaction
G6P:    -55.7   -0.4    -5.0    -100.0%
R5P:    1.5     0.4     6.0     119.9%
NADPH:  1.8     0.0     0.0     0.3%
ADP:    53.3    0.1     1.0     20.0%
CO2:    1.8     0.0     0.0     0.2%
H:      53.8    0.1     1.0     20.3%
H2O:    -0.1    -0.0    -0.0    -0.2%

 Path = NADPH
G6P:    -56.9   -0.1    -1.0    -100.0%
R5P:    0.1     0.0     0.0     0.0%
NADPH:  3.7     1.0     11.6    1155.1%
ADP:    53.2    -0.1    -1.0    -95.7%
CO2:    3.3     0.5     5.8     575.7%
H:      54.2    0.9     10.6    1059.4%
H2O:    -0.1    -0.5    -5.8    -576.2%
```

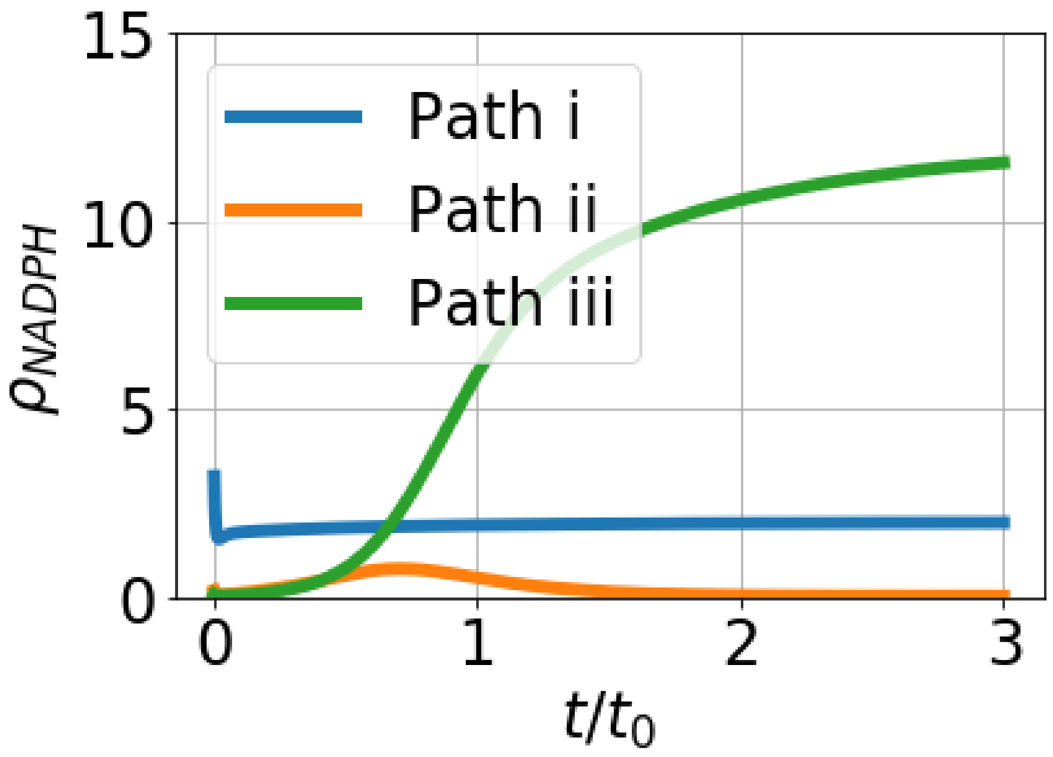
8.6 Plot ratios

```
[39]: ## Plot ratios
name = ['i','ii','iii']
for sp in ['R5P','NADPH']:
    BigFont = 24
    plt.rcParams.update({'font.size': BigFont})
    for i,path in enumerate(['both','R5P','NADPH']):
        Ratio = RATIO[path]
        label = f'Path {name[i]}'
        plt.plot(t/t_0,Ratio[sp],label=label,linewidth=5)
    ylabel = r'$\rho_{'+sp+'}$'
    plt.ylabel(ylabel)
    plt.xlabel('$t/t_0$')
    plt.legend()
    plt.grid()
    if sp=='R5P':
        ylim = 8
    else:
        ylim=15
    plt.ylim((0,ylim))

    if SaveFig:
        plt.savefig(f'Figs/{sp}_MM.pdf',bbox_inches='tight')

    plt.show()
```

```
[39]: [<matplotlib.lines.Line2D at 0x7fc316b906a0>]

[39]: [<matplotlib.lines.Line2D at 0x7fc316b90880>]

[39]: [<matplotlib.lines.Line2D at 0x7fc316d8c580>]

[39]: Text(0,0.5,'$\\rho_{R5P}$')

[39]: Text(0.5,0,'$t/t_0$')

[39]: <matplotlib.legend.Legend at 0x7fc31085e0d0>

[39]: (0, 8)
```



```
[39]: [<matplotlib.lines.Line2D at 0x7fc3107ed070>]

[39]: [<matplotlib.lines.Line2D at 0x7fc3107ed880>]

[39]: [<matplotlib.lines.Line2D at 0x7fc3107cdbb0>]

[39]: Text(0,0.5,'$\\rho_{NADPH}$')

[39]: Text(0.5,0,'$t/t_0$')

[39]: <matplotlib.legend.Legend at 0x7fc3107ed310>

[39]: (0, 15)
```



```
[40]: ## Compare concentrations
      conc_mam = Concentration['Mammalian']
      conc_eco = Concentration['Ecoli']
      for spec in conc_eco.keys():
          ratio = conc_eco[spec]/conc_mam[spec]
          print(f'{spec}: {ratio:2.2e}')
```

```
6PGC: 2.28e+02
ADP: 9.75e-01
ATP: 2.06e+00
CO2: 9.86e-03
DHAP: 1.88e+00
E4P: 4.76e+00
F6P: 2.60e+01
FDP: 1.00e+01
G3P: 1.92e+00
G6P: 1.17e+01
NADP: 7.32e-02
NADPH: 1.85e+00
R5P: 2.77e+01
RU5PD: 2.13e+01
S7P: 4.87e+01
XU5PD: 6.05e+00
```

```
[ ]:
```

```
[ ]:
```

References

Peter J Gawthrop. Energy-based Feedback Control of Biomolecular Systems with Cyclic Flow Modulation. Available at arXiv:2007.14762, July 2020.

Peter J. Gawthrop, Peter Cudmore, and Edmund J. Crampin. Physically-plausible modelling of biomolecular systems: A simplified, energy-based model of the mitochondrial electron transport chain. *Journal of Theoretical Biology*, 493: 110223, 2020. ISSN 0022-5193. doi: 10.1016/j.jtbi.2020.110223.

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*, 2010. doi: 10.1128/ecosalplus.10.2.1.

Junyoung O. Park, Sara A. Rubin, Yi-Fan Xu, Daniel Amador-Noguez, Jing Fan, Tomer Shlomi, and Joshua D. Rabinowitz. Metabolite concentrations, fluxes and free energies imply efficient enzyme usage. *Nat Chem Biol*, 12(7):482-489, Jul 2016. ISSN 1552-4450. doi: 10.1038/nchembio.2077.