Phosphorylation, Dephosphorylation and the Mitogen-activated Protein Kinase (MAPK) cascade

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Contents

Note: this is the Phosphorylation.ipynb notebook. The PDF version is available here.

1 Introduction

The Mitogen-activated Protein Kinase (MAPK) cascade is a well-studied signalling pathway with ultrasensitive components (Alberts et al., 2015; Klipp et al., 2016). However, the use of the Michaelis-Menten approximation to enzyme-catalysed reactions can be misleading in this context (Voit, 2013).

Following (Gawthrop and Crampin, 2016), each phosphorylation step is built out of reversible mass-action reactions using the bond graph approach of (Gawthrop and Crampin, 2014). This resolves the potential problems mentioned above as well as giving a thermodynamically compliant model which explicitly accounts for energy consumption via ATP hydrolysis.

This notebook presents, analyses and simulates three bond graph models using BondGraphTools and extensions:

1. A phosphorylation/dephosphorylation system (PD).
2. A double phosphorylation/dephosphorylation system (DPD).
3. A Mitogen-activated Protein Kinase (MAPK) cascade using a cascade of one PD systems and two DPD systems.

1.1 Import some python code

The bond graph analysis uses a number of Python modules:

```
In [1]: ## Some useful imports
        import BondGraphTools as bgt
        import numpy as np
        import sympy as sp
        import matplotlib.pyplot as plt
        import IPython.display as disp

        ## Stoichiometric analysis
        import stoich as st

        ## SVG bg representation conversion
        import svgBondGraph as sbg

        ## Modular bond graphs
        import modularBondGraph as mbg

        ## Export stoichiometry as bond graph
        import stoichBondGraph as stbg

        ## Data structure copy
        import copy
```

```
## Set quiet=False for verbose output
quiet = True
```

2 Phosphorylation/dephosphorylation

A biomolecular cycle involving both phosphorylation and dephosphorylation is a basic element of cell signalling (Alberts et al., 2015). A bond graph model of this cycle (Gawthrop and Crampin, 2014, 2016) is given in the Figure.

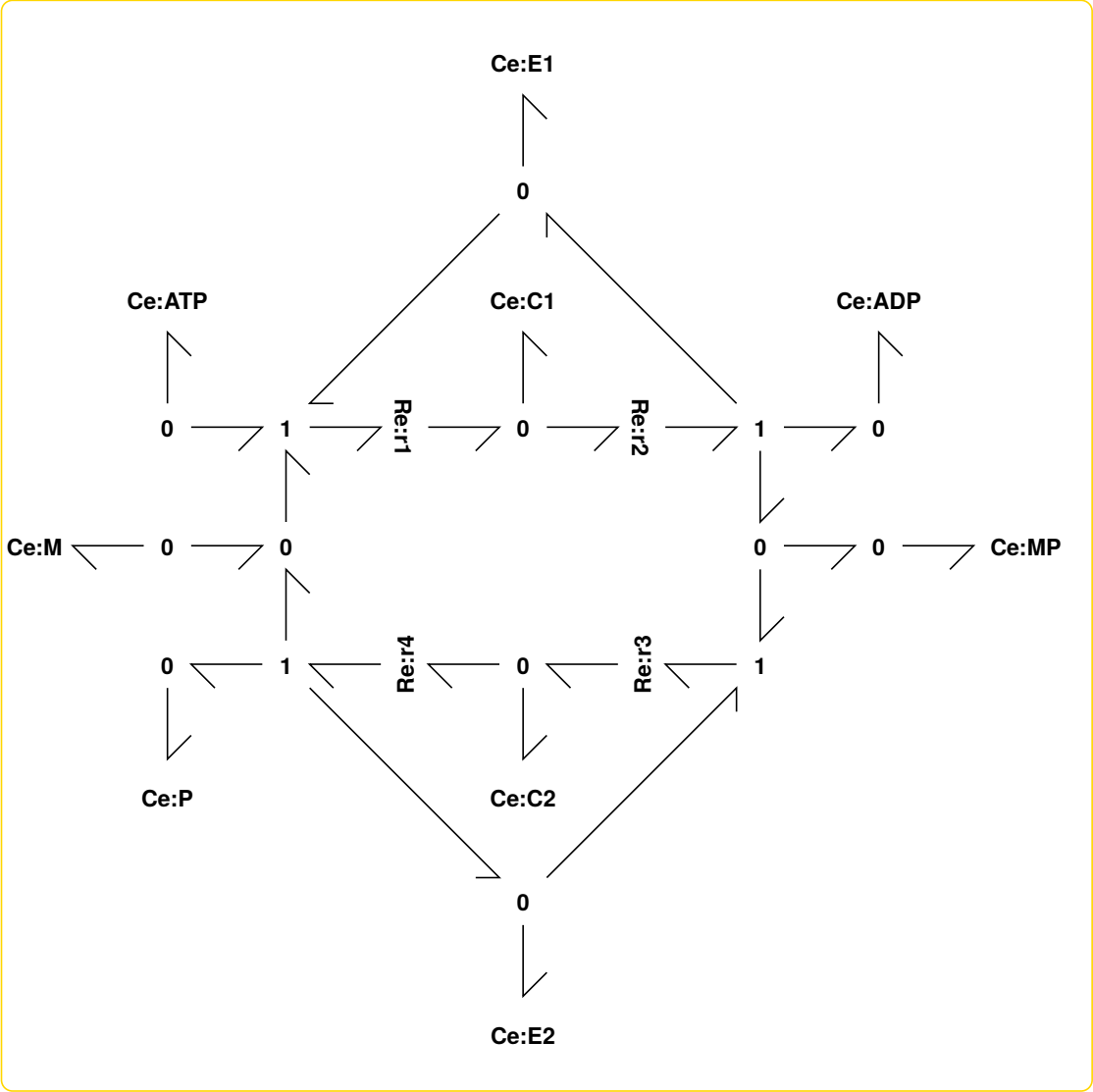
1. The upper part of the bond graph represents an enzyme-catalysed reaction phosphorylating protein M to give MP driven by the dephosphorylation of ATP to ADP.
2. The lower part of the bond graph represents an enzyme-catalysed reaction dephosphorylating protein MP to give M and P.

This forms a biochemical switch (Beard and Qian, 2010) with input the net amount of E1 and C1 and output the phosphorylated protein MP.

2.1 Convert bond graph in SVG format to BGT format and display

In [2]:
```
## Phosphorylation/dephosphorylation
sbg.model('PD_abg.svg',quiet=quiet)
import PD_abg
disp.SVG('PD_abg.svg')
```

Out[2]:



2.2 A flowstat is used to add to the pool of enzyme formed by E1 and C1

The flowstat is formed from a simple reaction A⇔B where A is a chemostat and B is unified with E1. The flow in this reaction is set in the simulation.

```
In [3]: def addFlowstat(model,species,quiet=False):

            ## Creat a simple reaction
            sbg.model('AB_abg.svg',quiet=quiet)
            import AB_abg
            AB = AB_abg.model()
            mbg.rename(AB,{'A':'Aflow','B':species},quiet=quiet)
```

```
        ## Create composite model
        modelF = bgt.new(name='modelF')
        modelF.add(model,AB)
        mbg.unify(modelF,[species],quiet=quiet)

        return modelF

PDF = addFlowstat(PD_abg.model(),'E1',quiet=quiet)
```

2.3 Reactions, pathways and pools

The coresponding reactions and pathways are generated using stoich.

1. The four reactions r1-r4 correspond to the four Re components in the bond graph.
2. The reaction r corresponds to the added flowstat: the flow in r is externally specified and provides a way to change the amount of enzyme in the E1/P1 pool.
3. There is a single pathway though the four components r1-r4 corresponding the the flow around the loop driven by the reaction ATP = ADP + P.
4. The two enzyme catalysed reactions are modulated by {E1} and E2 which therfore determine the flows and the relative amounts of M and MP.
5. Apart from the chemostats which are themselves conserved moieties, there are two pools:
   a) C2 + E2
   b) C1 + C2 + M + MP

Note that C1+E1 is not a pool due to the input flow from the flowstat.

2.3.1 Reactions

```
In [4]: s = st.stoich(PDF,quiet=quiet)
        disp.Latex(st.sprintrl(s,chemformula=True))
```

Out[4]:

$$\mathrm{ATP} + \mathrm{M} + \mathrm{E_1} \overset{\mathrm{r_1}}{\rightleftharpoons} \mathrm{C_1} \tag{1}$$

$$\mathrm{C_1} \overset{\mathrm{r_2}}{\rightleftharpoons} \mathrm{ADP} + \mathrm{MP} + \mathrm{E_1} \tag{2}$$

$$\mathrm{E_2} + \mathrm{MP} \overset{\mathrm{r_3}}{\rightleftharpoons} \mathrm{C_2} \tag{3}$$

$$\mathrm{C_2} \overset{\mathrm{r_4}}{\rightleftharpoons} \mathrm{E_2} + \mathrm{M} + \mathrm{P} \tag{4}$$

$$\mathrm{Aflow} \overset{\mathrm{r}}{\rightleftharpoons} \mathrm{E_1} \tag{5}$$

2.3.2 Pathways

The ATP hydrolysis species are set as chemostats together with Aflow from the flowstat reaction

```
In [5]: chemostats = ['Aflow','ATP','ADP','P']
        sc = st.statify(s,chemostats=chemostats)
        print(st.sprintp(sc))
```

```
1 pathways
0:  + r1 + r2 + r3 + r4
```

```
In [6]: sp = st.path(s,sc)
        disp.Latex(st.sprintrl(sp))
```

Out[6]:

$$ATP \Leftrightarrow ADP + P \tag{6}$$

2.3.3 Pools: Conserved moieties

```
In [7]: disp.Latex(st.sprintml(sc,chemformula=True))
```

Out[7]:

$$\xrightleftharpoons{m_0} \text{ADP} \tag{7}$$
$$\xrightleftharpoons{m_1} \text{ATP} \tag{8}$$
$$\xrightleftharpoons{m_2} \text{C}_2 + \text{E}_2 \tag{9}$$
$$\xrightleftharpoons{m_3} \text{C}_1 + \text{C}_2 + \text{M} + \text{MP} \tag{10}$$
$$\xrightleftharpoons{m_4} \text{P} \tag{11}$$
$$\xrightleftharpoons{m_5} \text{Aflow} \tag{12}$$

2.4 Set up parameters for PD

```
In [8]: def setParameterPD(s,x_M=1,x_E2=0.1):
            """Set up parameters and states for simulation of PD module"""

            parameter = {}

            # Ce components: set non-unity parameters
            K_ATP = 1e2
            K_ADP = 1e-3
            K_P = 1e-3
            parameter['K_ATP'] = K_ATP
```

```
        parameter['K_ADP'] = K_ADP
        parameter['K_P'] = K_P
        parameter['K_E1'] = 1
        parameter['K_E2'] = 1
        parameter['K_C1'] = 100
        parameter['K_C2'] = 100

        ## Initial states
        small = 1e-10

        ## Small initial values
        smallStates = ['E1','C1','C2','MP']
        for smallState in smallStates:
            parameter['X0_'+smallState] = small

        ## Initial values of other states
        parameter['X0_M'] = x_M
        parameter['X0_E2'] = x_E2

        return parameter
```

2.4.1 Plotting

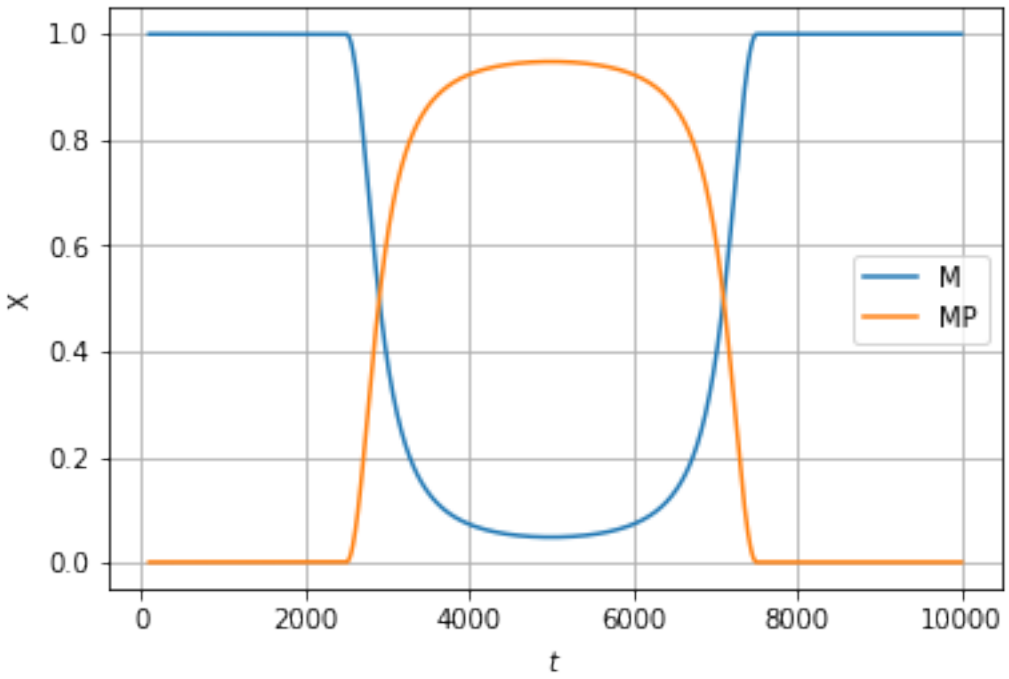
```
In [9]: def inPool(s,X,species):
        """Find total amount in pool specified by species"""

        index = []
        for spec in species:
            index.append(s['species'].index(spec))

        total = np.sum(X[:,index],axis=1)

        return total

    def Plot(s,dat,M=['M','MP'],E=['E1','C1'],i0=0):
        """Plot relevant data"""

        ## Extract data
        X = dat['X']
        V = dat['V']
        N = s['N']
        dX = (N@V.T).T
        dX_ATP = dX[:,s['spec_index']['ATP']]
        dX_ADP = dX[:,s['spec_index']['ADP']]
        dX_P = dX[:,s['spec_index']['P']]


        st.plot(s,dat,species=M,reaction = [],i0=10)
```

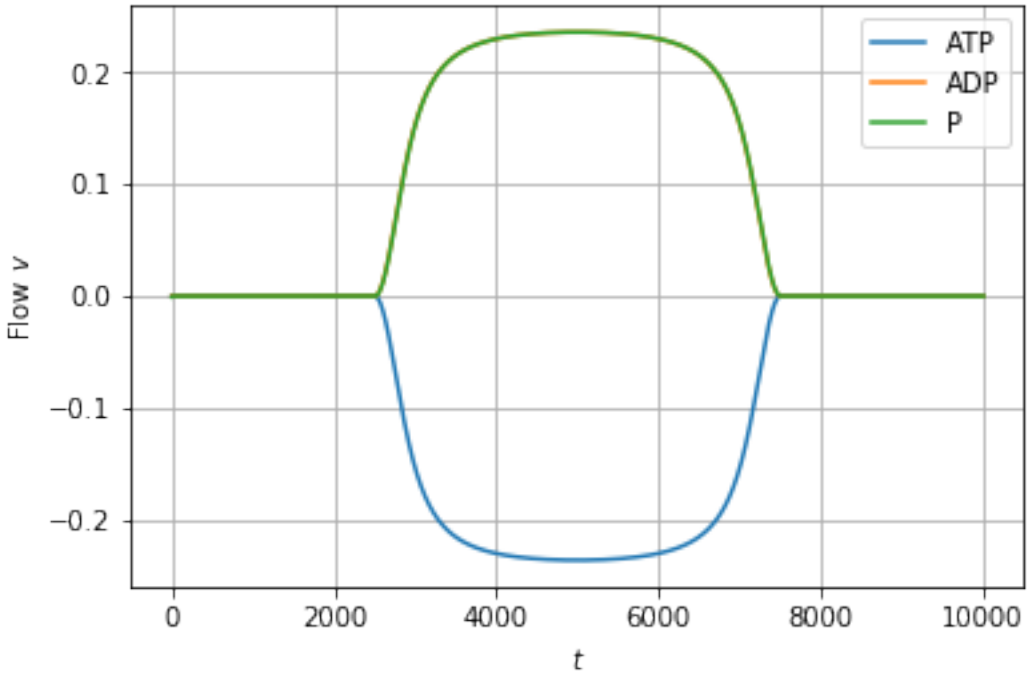
```
plt.plot(t,dX_ATP,t,dX_ADP,t,dX_P)
plt.grid()
plt.ylabel('Flow $v$')
plt.xlabel('$t$')
plt.legend(['ATP','ADP','P'])
plt.show()

e_tot = inPool(s,X,E)

plt.plot(t,e_tot)
plt.grid()
plt.xlabel('$t$')
plt.show()

st.plot(s,dat,species=M,reaction = [],x=e_tot,xlabel='$e_{tot}$',i0=10)
```

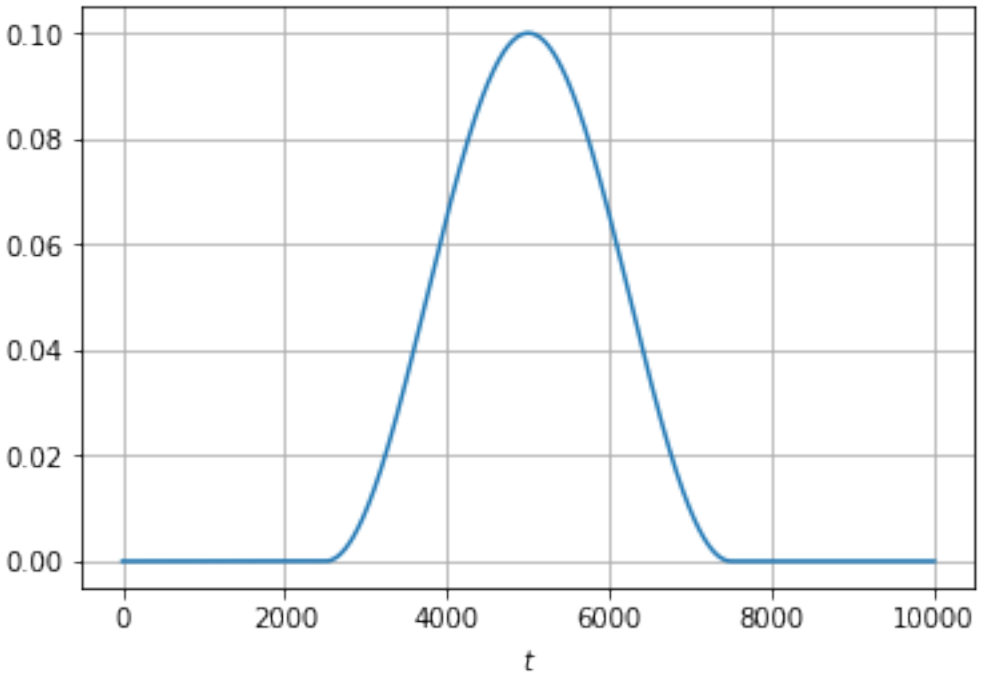
2.5 Simulation

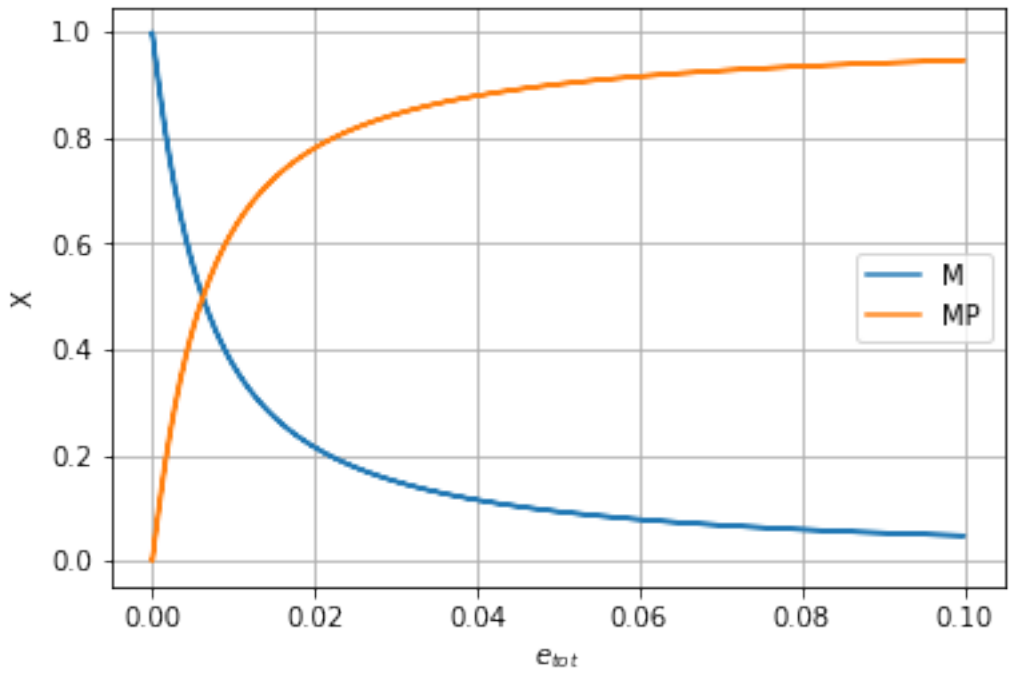
```
In [10]: def setFlow(e_max,t_max):
             """Set the flow stat flow (as a string)

             Flow is non-zero between 0.25 and 0.75 t_max
             and is sinusoidal.
             The integrated flow has a maximum at e_tot = e_max

             """
             r_flow = ('2*{0}*( (t>(0.25*{1}))*(t<(0.75*{1})) )'
                       '*np.sin(4*np.pi*(t-0.25*{1})/{1})'
                         .format(np.pi*e_max/(t_max),t_max)
                       )
             V_flow = {'r':r_flow}

             return V_flow


         ##Time
         quiet = True
         #t_max = int(8e2)
         t_max = 1e4
         t = np.linspace(0,t_max,1000)
         t_0 = 100
         t_1 = t_max-t_0
         i_max = len(t)
         i_0 = int(i_max*t_0/t_max)
         i_1 = i_max-i_0
```

```
## Flow
x_M = 1
e_max = 1e-1
V_flow = setFlow(e_max,t_max)

## Parameters
x_E2=0.5*x_M
parameter = setParameterPD(s,x_M=x_M,x_E2=x_E2)

## Simulate
dat = st.sim(s,sc=sc,t=t,parameter=parameter,V_flow=V_flow,quiet=quiet)

## Plot
Plot(s,dat,M=['M','MP'],E=['E1','C1'])
```




ATP
ADP
P
0.2
0.1
0.0
−0.1
−0.2
Flow v
0
2000
4000
6000
8000
10000
t


0.10
0.08
0.06
0.04
0.02
0.00
0
2000
4000
6000
8000
10000
t



2.6 Discussion

The PD module acts and a high-gain saturating amplifier, or switch (Beard and Qian, 2010), with the total enzyme associated with the first reaction $e_{tot} = x_{E1} + x_{c1}$ as the input and the amount of the phosphorylated protein MP as output. Note that e_{tot} is varied using the flowstat.

3 Double Phosphorylation/dephosphorylation

Double phosphorylation/dephosphorylation is an important building block of signalling cascades (Alberts et al., 2015; Klipp et al., 2016). A model can be built by combining two copes of the PD module using BondGraphTools (Cudmore et al., 2019).

3.1 Set up parameters for DPD

```
In [11]: def copyNames(names,same=[],changed={},prefix=''):

             rename = {}
             for name in names:
                 if not name in same:
                     if name in changed.keys():
                         rename[name] = changed[name]
                     else:
                         rename[name] = prefix+name
```

```
        return rename

    def copyParameters(parameter,rename):

        sep = '_' # parameter seperator
        Parameter = {}
        for key,val in parameter.items():
            Key = key.split(sep)
            #print(key,Key)
            if len(Key)<2:
                print(key,'should contain _')
            else:
                prefix = Key[0]
                name = Key[1]
                for nam in Key[2:]:
                    name += sep+nam

            if name not in rename.keys():
                Parameter[key] = val
            else:
                Parameter[prefix+sep+rename[name]] = val
        return Parameter

    def mergeParameters(par1,par2):

    #    par = par1.copy()
        par = {}
        for key,val in par2.items():
            par[key] = val
        for key,val in par1.items():
            par[key] = val
        return par
```

3.2 Create DPD from two copies of PD

```
In [12]: def makeDPD(x_M=x_M,x_E2=x_E2,quiet=False):
             """Create Double Phosphorylation/dephosphorylation"""


             ## Components not to be renamed
             same = ['E1','ATP','ADP','P']

             ## Common components to be unified
             unified = same + ['MP']

             ## Create two copies of PD, renaming as appropriate
             PD1 = PD_abg.model()
             sPD = st.stoich(PD_abg.model(),quiet=quiet)
```

```
PD1.name = 'PD1'

names = sPD['species'] + sPD['reaction']
rename = copyNames(names,prefix='PD1__',same=same+['M','MP'])
mbg.rename(PD1,rename,quiet=quiet)

## Parameters of PD
parameterPD = setParameterPD(sPD,x_M=x_M,x_E2=x_E2)

## Parameters of P1
parameter_P1 = copyParameters(parameterPD,rename)

PD2 = PD_abg.model()
PD2.name = 'PD2'
rename = copyNames(names,prefix='PD2__',same=same, changed={'M':'MP_','MP':'MPP'})
mbg.rename(PD2,rename,quiet=quiet)
mbg.rename(PD2,{'MP_':'MP'},quiet=quiet)

## Parameters of P2
parameter_P2 = copyParameters(parameterPD,rename)

## DPD parameters
parameter_DPD = mergeParameters(parameter_P1,parameter_P2)

## Create DPD
DPD = bgt.new(name='DPD')
DPD.add(PD1,PD2)

## Unify common species
mbg.unify(DPD,unified,quiet=quiet)

## Stoichiometry of DPD
sDPD = st.stoich(DPD,quiet=quiet)

## Save as flattened bond graph for later use
sDPD['name'] = 'DPD_abg'
stbg.model(sDPD)

## Add in the flowstat
DPDF = addFlowstat(DPD,'E1',quiet=quiet)

## Stoichiometry
s = st.stoich(DPDF,quiet=quiet)
chemostats = ['Aflow','ATP','ADP','P']
sc = st.statify(s,chemostats=chemostats)

return s,sc,parameter_DPD,sPD,sDPD
```

```
x_E2 = 0.1*x_M
S,Sc,Parameter,sPD,sDPD = makeDPD(x_M=x_M,x_E2=x_E2,quiet=quiet)
```

3.3 Reactions, pathways and pools

The dot (.) notation is used to represent species and reactions within each submodule. Thus PD1.C1 and PD2.C2 represent the C2 species associated with each submodule and PD1.r1 and PD2.r1 reaction r1 associated with each submodule.

1. There is a pathway though the four components r1-r4 within each submodule corresponding the the flow around the loop driven by the reaction ATP = ADP + P.
2. Apart from the chemostats which are themselves conserved moieties, there are three pools:
   a) PD1.C2 + PD1.E2
   b) PD2.C2 + PD2.E2
   c) PD1.C1 + PD1.C2 + PD2.C1 + PD2.C2 + M + MP + MPP

3.3.1 Reactions

```
In [13]: disp.Latex(st.sprintrl(S,chemformula = True))
```

Out[13]:

$$\mathrm{M} + \mathrm{ATP} + \mathrm{E_1} \xrightleftharpoons{\mathrm{PD_1 \cdot r_1}} \mathrm{PD_1 \cdot C_1} \tag{13}$$

$$\mathrm{PD_1 \cdot C_1} \xrightleftharpoons{\mathrm{PD_1 \cdot r_2}} \mathrm{ADP} + \mathrm{MP} + \mathrm{E_1} \tag{14}$$

$$\mathrm{PD_1 \cdot E_2} + \mathrm{MP} \xrightleftharpoons{\mathrm{PD_1 \cdot r_3}} \mathrm{PD_1 \cdot C_2} \tag{15}$$

$$\mathrm{PD_1 \cdot C_2} \xrightleftharpoons{\mathrm{PD_1 \cdot r_4}} \mathrm{PD_1 \cdot E_2} + \mathrm{M} + \mathrm{P} \tag{16}$$

$$\mathrm{ATP} + \mathrm{MP} + \mathrm{E_1} \xrightleftharpoons{\mathrm{PD_2 \cdot r_1}} \mathrm{PD_2 \cdot C_1} \tag{17}$$

$$\mathrm{PD_2 \cdot C_1} \xrightleftharpoons{\mathrm{PD_2 \cdot r_2}} \mathrm{MPP} + \mathrm{ADP} + \mathrm{E_1} \tag{18}$$

$$\mathrm{PD_2 \cdot E_2} + \mathrm{MPP} \xrightleftharpoons{\mathrm{PD_2 \cdot r_3}} \mathrm{PD_2 \cdot C_2} \tag{19}$$

$$\mathrm{PD_2 \cdot C_2} \xrightleftharpoons{\mathrm{PD_2 \cdot r_4}} \mathrm{PD_2 \cdot E_2} + \mathrm{P} + \mathrm{MP} \tag{20}$$

$$\mathrm{Aflow} \xrightleftharpoons{\mathrm{r}} \mathrm{E_1} \tag{21}$$

3.3.2 Pathways

```
In [14]: print(st.sprintp(Sc))
         Sp = st.path(S,Sc)
         disp.Latex(st.sprintrl(Sp,chemformula = True))
```

```
2 pathways
0:  + PD1.r1 + PD1.r2 + PD1.r3 + PD1.r4
1:  + PD2.r1 + PD2.r2 + PD2.r3 + PD2.r4
```

Out[14]:

$$\mathrm{ATP} \xrightleftharpoons{\mathrm{pr}_1} \mathrm{ADP} + \mathrm{P} \tag{22}$$

$$\mathrm{ATP} \xrightleftharpoons{\mathrm{pr}_2} \mathrm{ADP} + \mathrm{P} \tag{23}$$

3.3.3 Pools

In [15]:
```
disp.Latex(st.sprintml(Sc,chemformula=True))
```

Out[15]:

$$\xrightleftharpoons{\mathrm{m}_0} \mathrm{PD}_1\cdot\mathrm{C}_2 + \mathrm{PD}_1\cdot\mathrm{E}_2 \tag{24}$$

$$\xrightleftharpoons{\mathrm{m}_1} \mathrm{PD}_2\cdot\mathrm{C}_2 + \mathrm{PD}_2\cdot\mathrm{E}_2 \tag{25}$$

$$\xrightleftharpoons{\mathrm{m}_2} \mathrm{ATP} \tag{26}$$

$$\xrightleftharpoons{\mathrm{m}_3} \mathrm{ADP} \tag{27}$$

$$\xrightleftharpoons{\mathrm{m}_4} \mathrm{P} \tag{28}$$

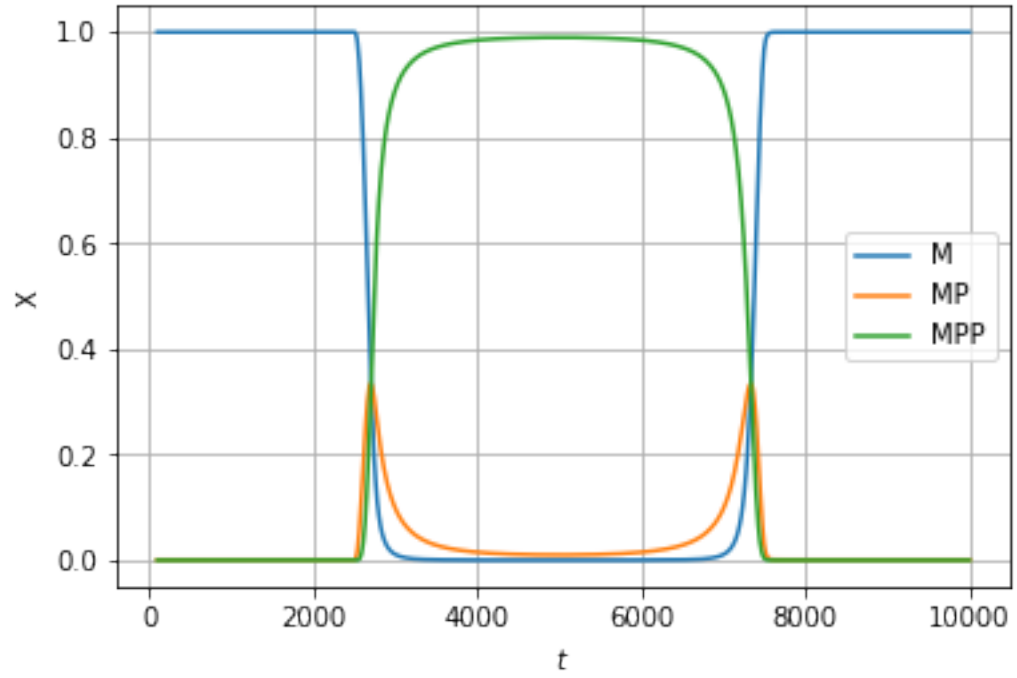
$$\xrightleftharpoons{\mathrm{m}_5} \mathrm{PD}_1\cdot\mathrm{C}_1 + \mathrm{PD}_1\cdot\mathrm{C}_2 + \mathrm{M} + \mathrm{PD}_2\cdot\mathrm{C}_1 + \mathrm{PD}_2\cdot\mathrm{C}_2 + \mathrm{MPP} + \mathrm{MP} \tag{29}$$

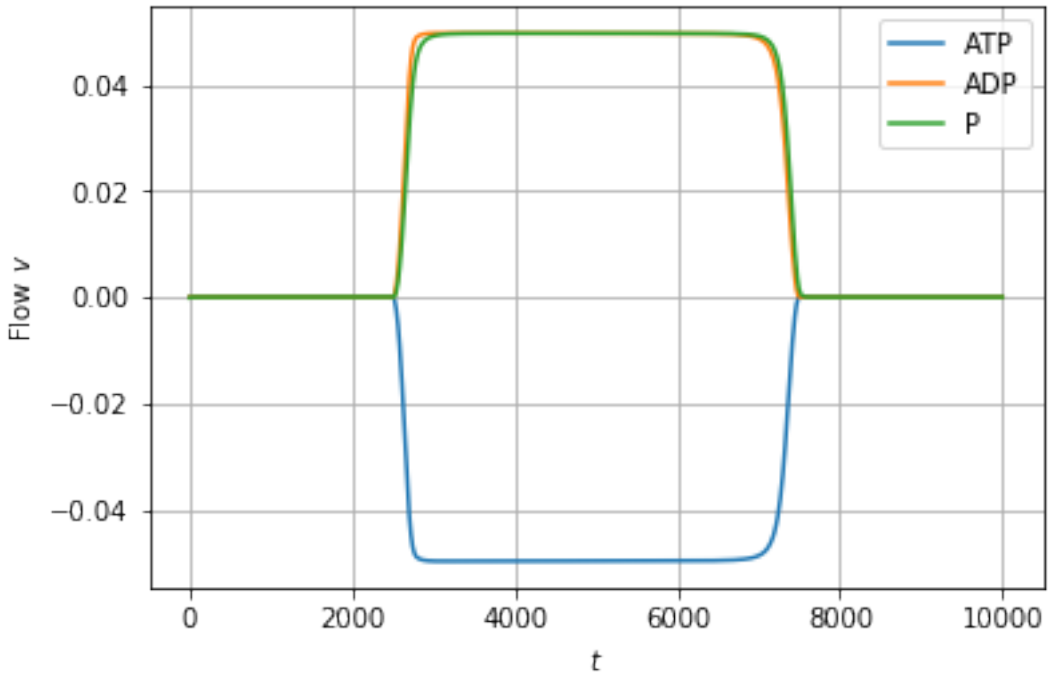
$$\xrightleftharpoons{\mathrm{m}_6} \mathrm{Aflow} \tag{30}$$

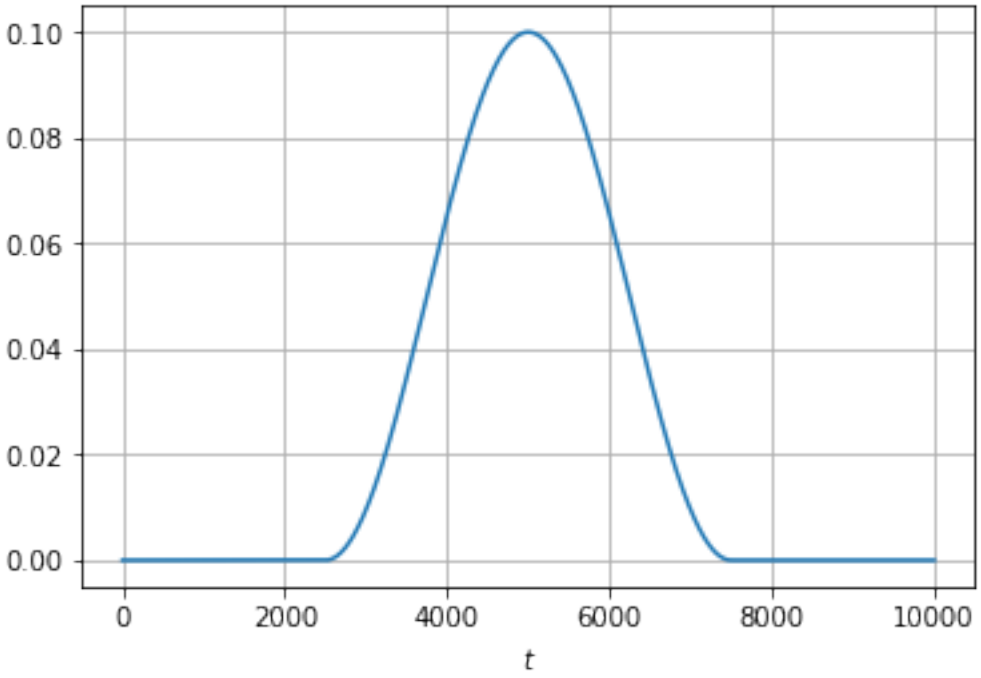
3.4 Simulation

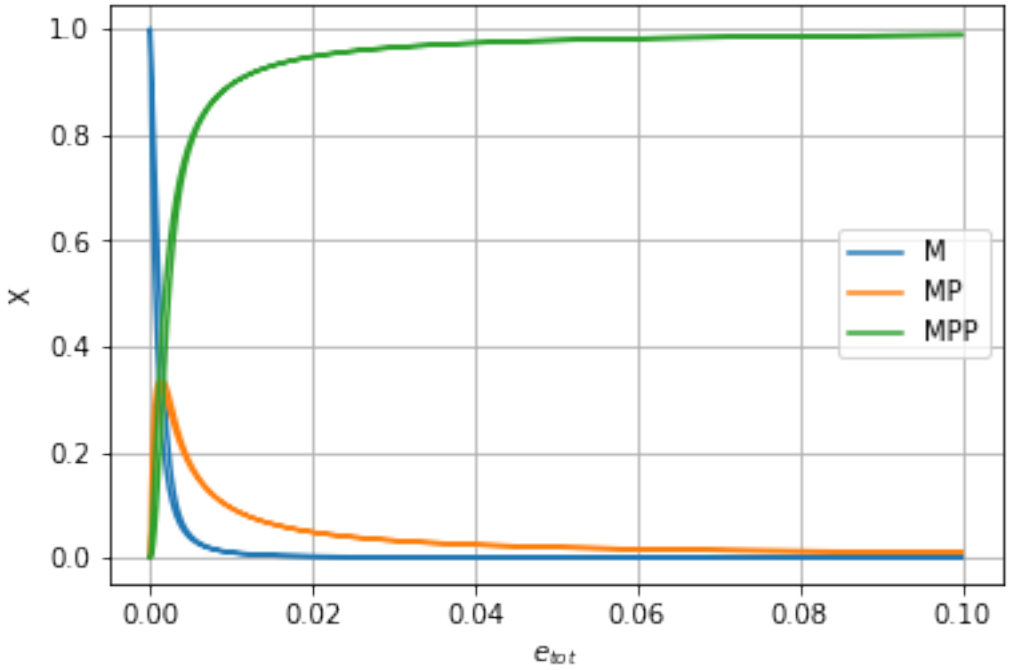
In [16]:
```
## Simulation

# Copy parameters and states to each sub module
# common = ['E1','M','MP','ATP','ADP','P','Aflow']


## Simulate
Dat = st.sim(S,sc=Sc,t=t,parameter=Parameter,V_flow=V_flow,quiet=quiet)

## Plot
Plot(S,Dat,M=['M','MP','MPP'],E=['E1','PD1__C1','PD2__C1'])
```

```
Unused parameters: ['X0_MP_']
```


1.0
0.8
0.6
0.4
0.2
0.0
X
0
2000
4000
6000
8000
10000
t
M
MP
MPP


0.04
0.02
0.00
−0.02
−0.04
Flow v
0
2000
4000
6000
8000
10000
t
ATP
ADP
P


0.10
0.08
0.06
0.04
0.02
0.00
0
2000
4000
6000
8000
10000
t


1.0
0.8
0.6
0.4
0.2
0.0
X
0.00
0.02
0.04
0.06
0.08
0.10
e_{tot}
M
MP
MPP

3.5 Discussion

In a similar way to the PD module, the DPD module acts and a high-gain saturating amplifier, or switch (Beard and Qian, 2010), with the total enzyme associated with the first reaction of the first PD $e_{tot} = x_{E1} + x_{c1}$ as the input and the amount of the double-phosphorylated protein MPP as output. Note that the gain is giher, and the behavior more switch-like compared to the PD module.

4 MAPK cascade

4.1 Create cascade from one PD and two DPD

```
In [17]: def makeMAPK(sPD,S,Parameter,useDPD=True,quiet=quiet):
             """Create the MAPK cascade"""

             ## Components not to be renamed
             same = ['ATP','ADP','P']

             ## Amount of M in each layer
             X_M = np.array([1,7,50])
             X_E2 = 0.5*X_M

             ## Phosphorylation layer 1
             names = sPD['species'] + sPD['reaction']
             P1 = PD_abg.model()
             P1.name = 'P1'
             rename = copyNames(names,prefix='L1__',same=same,
                                changed={'M':'MKKK','MP':'MKKKP','E1':'MKKKK'})
             mbg.rename(P1,rename,quiet=quiet)
             parameter_P1 = copyParameters(setParameterPD(s,x_M=X_M[0],x_E2=X_E2[0]),rename)

             if not useDPD:
                 ## Use PD in place of DPD
                 ## Phosphorylation layer 2
                 names = sPD['species'] + sPD['reaction']
                 P2 = PD_abg.model()
                 P2.name = 'P2'
                 rename = copyNames(names,prefix='L2__',same=same,
                                    changed={'M':'MKK','MP':'MKKP','E1':'MKKKP'})
                 mbg.rename(P2,rename,quiet=quiet)
                 parameter_P2 = copyParameters(setParameterPD(s,x_M=X_M[1],x_E2=X_E2[1]),rename)

                 ## Phosphorylation layer 3
                 P3 = PD_abg.model()
                 P3.name = 'P3'
                 rename = copyNames(names,prefix='L3__',same=same,
                                    changed={'M':'MK','MP':'MKP','E1':'MKKP'})
                 mbg.rename(P3,rename,quiet=quiet)
```

```
    parameter_P3 = copyParameters(setParameterPD(s,x_M=X_M[2],x_E2=X_E2[2]),rename)

    connections = ['MKKKK','MKKKP','MKKP']
else:
## Use DPD
    import DPD_abg
    ## Phosphorylation layer 2
    S,Sc,Parameter,sPD,sDPD = makeDPD(x_M=X_M[1],x_E2=X_E2[1],quiet=quiet)
    names = sDPD['species'] + sDPD['reaction']
    P2 = DPD_abg.model()
    P2.name = 'P2'
    rename = copyNames(names,prefix='L2__',same=same,
                       changed={'M':'MKK','MP':'MKKP','MPP':'MKKPP','E1':'MKKKP'})
    mbg.rename(P2,rename,quiet=quiet)
    parameter_P2 = copyParameters(Parameter,rename)

    ## Phosphorylation layer 3
    S,Sc,Parameter,sPD,sDPD = makeDPD(x_M=X_M[2],x_E2=X_E2[2],quiet=quiet)
    names = sDPD['species'] + sDPD['reaction']
    print(names)
    P3 = DPD_abg.model()
    P3.name = 'P3'
    rename = copyNames(names,prefix='L3__',same=same,
                       changed={'M':'MK','MP':'MKP','MPP':'MKPP','E1':'MKKPP'})
    mbg.rename(P3,rename,quiet=quiet)
    parameter_P3 = copyParameters(Parameter,rename)

    connections = ['MKKKK','MKKKP','MKKPP']

## Flowstat
import AB_abg
AB = AB_abg.model()
mbg.rename(AB,{'A':'Aflow','B':'MKKKK'},quiet=quiet)

## Create the MAPK cascade with flowstat
MAPK = bgt.new(name='MAPK')
MAPK.add(AB,P1,P2,P3)
unify = same + connections
mbg.unify(MAPK,unify,quiet=quiet)

parameter_P12 = mergeParameters(parameter_P1,parameter_P2)
parameterM = mergeParameters(parameter_P12,parameter_P3)

## Stoichiometry
sM = st.stoich(MAPK,quiet=quiet)
chemostats = ['Aflow','ATP','ADP','P']
scM = st.statify(sM,chemostats=chemostats)
```

```
        return sM,scM,parameterM

    useDPD = True
    sM,scM,ParameterM = makeMAPK(sPD,S,Parameter,useDPD=useDPD,quiet=True)
```

```
['PD1__C1', 'PD1__C2', 'PD1__E2', 'M', 'PD2__C1', 'PD2__C2', 'PD2__E2', 'MPP', 'E1', 'ATP', 'ADP
```

4.2 Reactions, pathways and pools

The dot (.) notation is used to represent species and reactions within each submodule. Thus L2.PD1.C1 and L2.PD2.C2 represent the C2 species associated with each submodule within level 2 and L2.PD1.r1 and L2.PD2.r1 represent reaction r1 associated with each submodule within level 2.

1. There is a pathway though the four components r1-r4 within each of the five submodule corresponding the the flow around the loop driven by the reaction ATP = ADP + P.
2. Apart from the chemostats which are themselves conserved moieties, there are eight pools:
   a) C2 and E2 within each of the 5 submodules
   b) L1.C1 + L1.C2 + L2.PD1.C1 + L2.PD2.C1 + MKKK + MKKKP
   c) L3.PD1.C1 + L3.PD1.C2 + L3.PD2.C1 + L3.PD2.C2 + MKPP + MKP + MK
   d) L2.PD1.C1 + L2.PD1.C2 + L2.PD2.C1 + L2.PD2.C2 + L3.PD1.C1 + L3.PD2.C1 + MKKPP + MKKP + MKK

4.2.1 Reactions

```
In [18]: disp.Latex(st.sprintrl(sM,chemformula = True))
```

Out[18]:

$$\text{Aflow} \xrightleftharpoons{r} \text{MKKKK} \tag{31}$$

$$\text{MKKK} + \text{ATP} + \text{MKKKK} \xrightleftharpoons{L_1 \cdot r_1} L_1 \cdot C_1 \tag{32}$$

$$L_1 \cdot C_1 \xrightleftharpoons{L_1 \cdot r_2} \text{ADP} + \text{MKKKK} + \text{MKKKP} \tag{33}$$

$$L_1 \cdot E_2 + \text{MKKKP} \xrightleftharpoons{L_1 \cdot r_3} L_1 \cdot C_2 \tag{34}$$

$$L_1 \cdot C_2 \xrightleftharpoons{L_1 \cdot r_4} L_1 \cdot E_2 + \text{MKKK} + \text{P} \tag{35}$$

$$\text{MKK} + \text{ATP} + \text{MKKKP} \xrightleftharpoons{L_2 \cdot PD_1 \cdot r_1} L_2 \cdot PD_1 \cdot C_1 \tag{36}$$

$$L_2 \cdot PD_1 \cdot C_1 \xrightleftharpoons{L_2 \cdot PD_1 \cdot r_2} \text{MKKP} + \text{ADP} + \text{MKKKP} \tag{37}$$

$$L_2 \cdot PD_1 \cdot E_2 + \text{MKKP} \xrightleftharpoons{L_2 \cdot PD_1 \cdot r_3} L_2 \cdot PD_1 \cdot C_2 \tag{38}$$

$$L_2 \cdot PD_1 \cdot C_2 \xrightleftharpoons{L_2 \cdot PD_1 \cdot r_4} L_2 \cdot PD_1 \cdot E_2 + \text{MKK} + \text{P} \tag{39}$$

$$\text{MKKP} + \text{ATP} + \text{MKKKP} \xrightleftharpoons{L_2 \cdot PD_2 \cdot r_1} L_2 \cdot PD_2 \cdot C_1 \tag{40}$$

$$L_2 \cdot PD_2 \cdot C_1 \xrightleftharpoons{L_2 \cdot PD_2 \cdot r_2} ADP + MKKKP + MKKPP \tag{41}$$

$$L_2 \cdot PD_2 \cdot E_2 + MKKPP \xrightleftharpoons{L_2 \cdot PD_2 \cdot r_3} L_2 \cdot PD_2 \cdot C_2 \tag{42}$$

$$L_2 \cdot PD_2 \cdot C_2 \xrightleftharpoons{L_2 \cdot PD_2 \cdot r_4} L_2 \cdot PD_2 \cdot E_2 + MKKP + P \tag{43}$$

$$MK + ATP + MKKPP \xrightleftharpoons{L_3 \cdot PD_1 \cdot r_1} L_3 \cdot PD_1 \cdot C_1 \tag{44}$$

$$L_3 \cdot PD_1 \cdot C_1 \xrightleftharpoons{L_3 \cdot PD_1 \cdot r_2} MKP + ADP + MKKPP \tag{45}$$

$$L_3 \cdot PD_1 \cdot E_2 + MKP \xrightleftharpoons{L_3 \cdot PD_1 \cdot r_3} L_3 \cdot PD_1 \cdot C_2 \tag{46}$$

$$L_3 \cdot PD_1 \cdot C_2 \xrightleftharpoons{L_3 \cdot PD_1 \cdot r_4} L_3 \cdot PD_1 \cdot E_2 + MK + P \tag{47}$$

$$MKP + ATP + MKKPP \xrightleftharpoons{L_3 \cdot PD_2 \cdot r_1} L_3 \cdot PD_2 \cdot C_1 \tag{48}$$

$$L_3 \cdot PD_2 \cdot C_1 \xrightleftharpoons{L_3 \cdot PD_2 \cdot r_2} MKPP + ADP + MKKPP \tag{49}$$

$$L_3 \cdot PD_2 \cdot E_2 + MKPP \xrightleftharpoons{L_3 \cdot PD_2 \cdot r_3} L_3 \cdot PD_2 \cdot C_2 \tag{50}$$

$$L_3 \cdot PD_2 \cdot C_2 \xrightleftharpoons{L_3 \cdot PD_2 \cdot r_4} L_3 \cdot PD_2 \cdot E_2 + MKP + P \tag{51}$$

4.2.2 Pathways

```
In [19]: print(st.sprintp(scM))
         sMp = st.path(sM,scM)
         disp.Latex(st.sprintrl(sMp,chemformula = True))
```

```
5 pathways
0:  + L1.r1 + L1.r2 + L1.r3 + L1.r4
1:  + L2.PD1.r1 + L2.PD1.r2 + L2.PD1.r3 + L2.PD1.r4
2:  + L2.PD2.r1 + L2.PD2.r2 + L2.PD2.r3 + L2.PD2.r4
3:  + L3.PD1.r1 + L3.PD1.r2 + L3.PD1.r3 + L3.PD1.r4
4:  + L3.PD2.r1 + L3.PD2.r2 + L3.PD2.r3 + L3.PD2.r4
```

Out[19]:

$$ATP \xrightleftharpoons{pr_1} ADP + P \tag{52}$$

$$ATP \xrightleftharpoons{pr_2} ADP + P \tag{53}$$

$$ATP \xrightleftharpoons{pr_3} ADP + P \tag{54}$$

$$ATP \xrightleftharpoons{pr_4} ADP + P \tag{55}$$

$$ATP \xrightleftharpoons{pr_5} ADP + P \tag{56}$$

4.2.3 Pools

```
In [20]: disp.Latex(st.sprintml(scM,chemformula=True))
```

Out[20]:

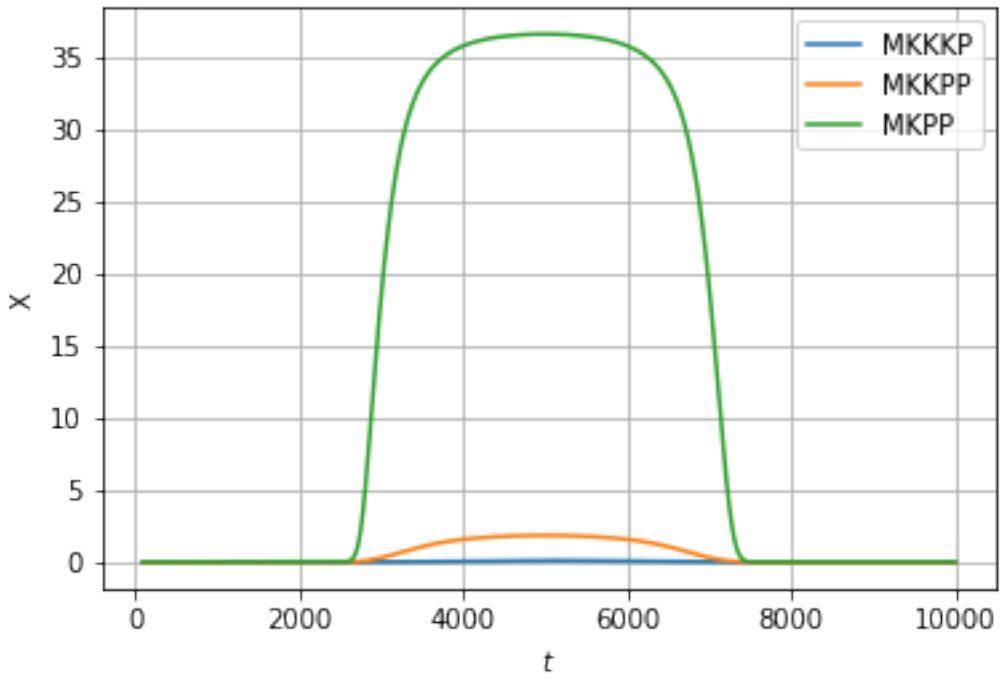
$$\xrightleftharpoons{m_0} \text{Aflow} \tag{57}$$
$$\xrightleftharpoons{m_1} L_1 \cdot C_2 + L_1 \cdot E_2 \tag{58}$$
$$\xrightleftharpoons{m_2} L_2 \cdot PD_1 \cdot C_2 + L_2 \cdot PD_1 \cdot E_2 \tag{59}$$
$$\xrightleftharpoons{m_3} L_2 \cdot PD_2 \cdot C_2 + L_2 \cdot PD_2 \cdot E_2 \tag{60}$$
$$\xrightleftharpoons{m_4} L_3 \cdot PD_1 \cdot C_2 + L_3 \cdot PD_1 \cdot E_2 \tag{61}$$
$$\xrightleftharpoons{m_5} L_3 \cdot PD_2 \cdot C_2 + L_3 \cdot PD_2 \cdot E_2 \tag{62}$$
$$\xrightleftharpoons{m_6} L_3 \cdot PD_1 \cdot C_1 + L_3 \cdot PD_1 \cdot C_2 + MK + L_3 \cdot PD_2 \cdot C_1 + L_3 \cdot PD_2 \cdot C_2 + MKPP + MKP \tag{63}$$
$$\xrightleftharpoons{m_7} \text{ATP} \tag{64}$$
$$\xrightleftharpoons{m_8} \text{ADP} \tag{65}$$
$$\xrightleftharpoons{m_9} P \tag{66}$$

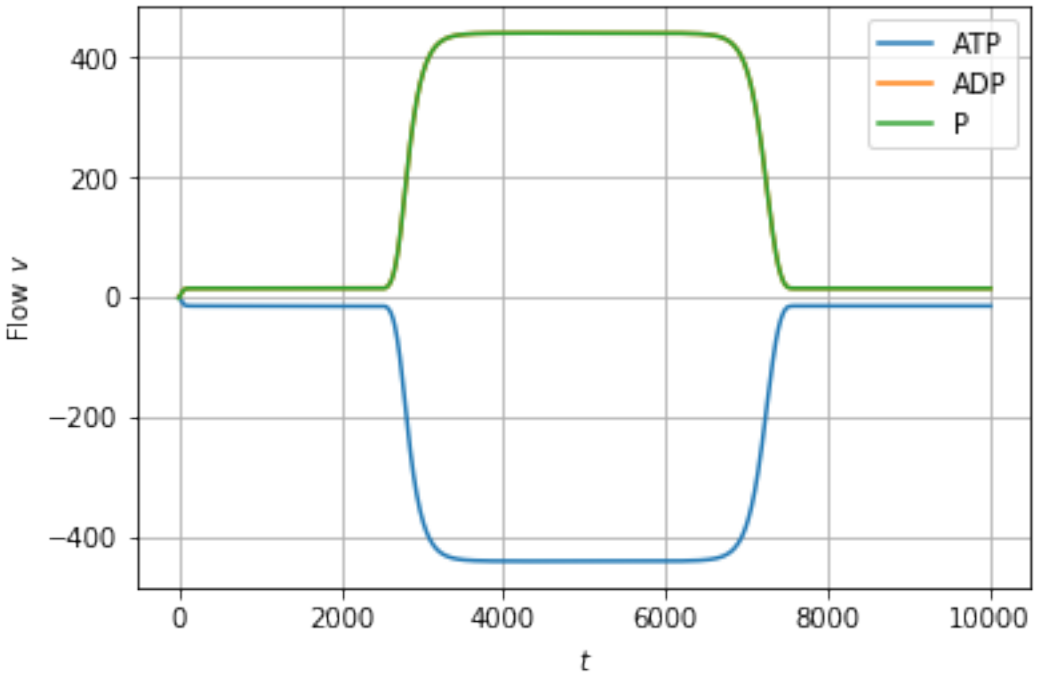
$$\xrightleftharpoons{m_{10}} L_1 \cdot C_1 + L_1 \cdot C_2 + MKKK + L_2 \cdot PD_1 \cdot C_1 + L_2 \cdot PD_2 \cdot C_1 + MKKKP \tag{67}$$
$$\xrightleftharpoons{m_{11}} L_2 \cdot PD_1 \cdot C_1 + L_2 \cdot PD_1 \cdot C_2 + MKK + L_2 \cdot PD_2 \cdot C_1 + L_2 \cdot PD_2 \cdot C_2 + MKKP + L_3 \cdot PD_1 \cdot C_1 + L_3 \cdot PD_2 \cdot C_1 + MK \tag{68}$$

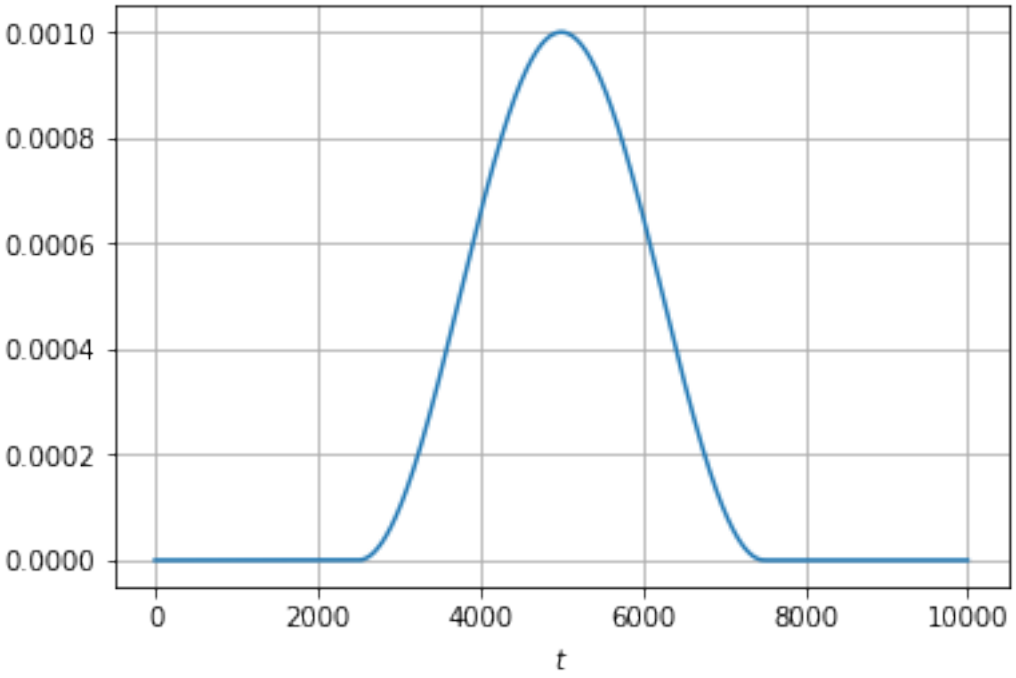
4.3 Simulation

```
In [21]: ## Flows
         e_max = 1e-3
         V_flow = setFlow(e_max,t_max)

         ## Simulate
         Dat = st.sim(sM,sc=scM,t=t,parameter=ParameterM,V_flow=V_flow,quiet=quiet)

         ## Plot
         if useDPD:
             Plot(sM,Dat,M=['MKKKP','MKKPP','MKPP'],E=['MKKKK','L1__C1'])
         else:
             Plot(sM,Dat,M=['MKKKP','MKKP','MKP'],E=['MKKKK','L1__C1'])
```

```
Unused parameters: ['X0_MP_']
```


MKKKP
MKKPP
MKPP
X
t
0
5
10
15
20
25
30
35
0
2000
4000
6000
8000
10000


ATP
ADP
P
Flow v
t
400
200
0
−200
−400
0
2000
4000
6000
8000
10000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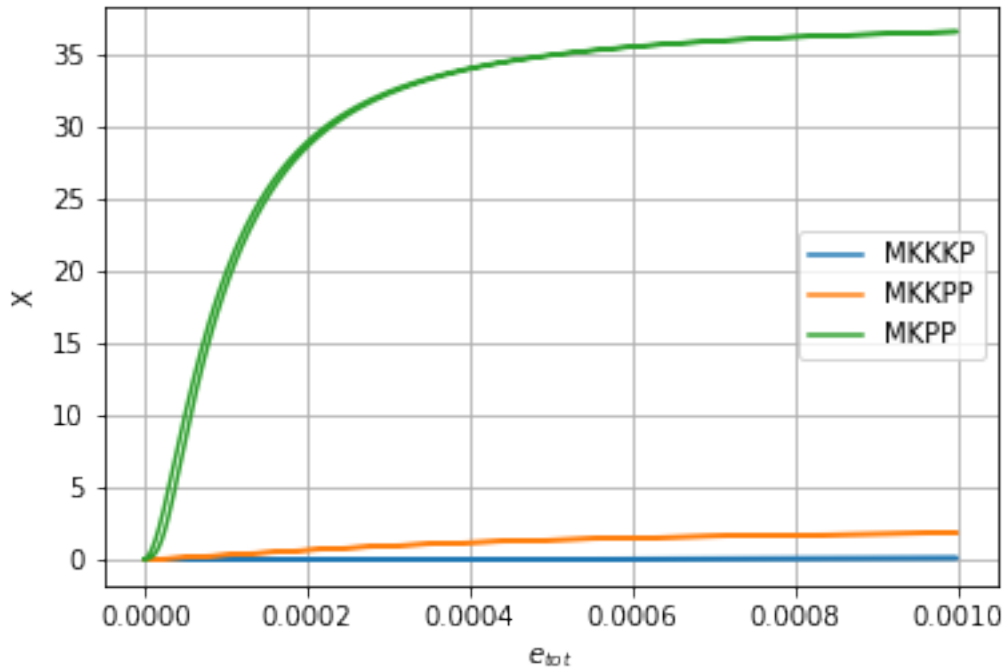
0.0008
0.0006
0.0004
0.0002
0.0000
0
2000
4000
6000
8000
10000
t


35
30
25
20
15
10
5
0
X
0.0000
0.0002
0.0004
0.0006
0.0008
0.0010
e_{tot}
MKKKP
MKKPP
MKPP

4.4 Discussion

In a similar way to the PD and DPD modules, the MAPK cascade module acts and a high-gain saturating amplifier, or switch with the total enzyme associated with the first reaction of the PD $e_{tot} = x_{E1} + x_{c1}$ of the first layer as the input and the amount of the double-phosphorylated protein MKPP of the third layer as output. Here, the maximum value (0.001) of the input is 100 times smaller than that of the simulations of PD and DPD and so the gain is much higher, and the behavior more switch-like compared to the PD and DPD modules.

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