Modulated Cooperative Enzyme-catalysed Reactions

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Contents

Note: this is the modulatedCooperative.ipynb notebook. The PDF version "Modulated Cooperative Enzyme-catalysed Reactions" is available here.

1 Introduction

"the ordinary laws [Michaelis-Menten] are inadequate for supplying the degree of control needed for metabolism" (Cornish-Bowden, 2013). In fact, key metabolic enzymes display *cooperativity* which *"display the property of responding with exceptional sensitivity to changes in metabolite concentrations"* (Cornish-Bowden, 2013).

Cooperativity is discussed in the notebook Cooperativity. Here, the cooperativity is augmented by *competitive activation and inhibition* to provide a mechanism for modulation and hence feedback control.

This note gives a bond graph (Gawthrop and Crampin, 2014) interpretation of such modulated cooperativity and uses the iterative properties of BondGraphTools (Cudmore et al., 2019) to build high-order modulated cooperative 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

        ## Data structure copy
        import copy

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

2 Modulated Cooperative Enzyme-catalysed Reaction

(Keener and Sneyd, 2009), Section 1.4.4, discusses cooperativity. This section gives a bond graph interpretation. This is done in two ways:

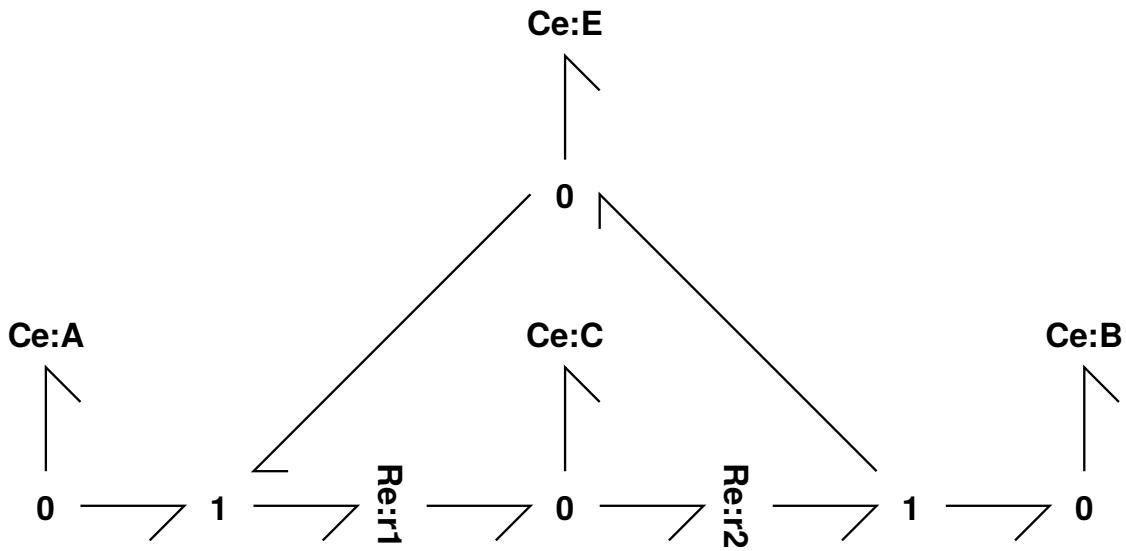
1. As a graphical representation of a two-stage cooperative enzyme-catalysed reaction.
2. As a generic representation of an N-stage cooperative enzyme-catalysed reaction using bond-graph tools

2.1 Enzyme-catalysed reaction

The basic enzyme-catalysed reaction is given in this section. It is the basic building block of cooperative enzyme-catalysed reactions More details are given by (Gawthrop and Crampin, 2014).

```
In [2]: ## Enzyme-catalysed reaction
        sbg.model('RE_abg.svg')
        import RE_abg
        disp.SVG('RE_abg.svg')
```

Out[2]:




```
In [3]: s = st.stoich(RE_abg.model(),quiet=quiet)
        disp.Latex(st.sprintrl(s,chemformula=True))
```

Out[3]:

$$\mathrm{A} + \mathrm{E} \underset{}{\overset{r_1}{\rightleftharpoons}} \mathrm{C} \tag{1}$$

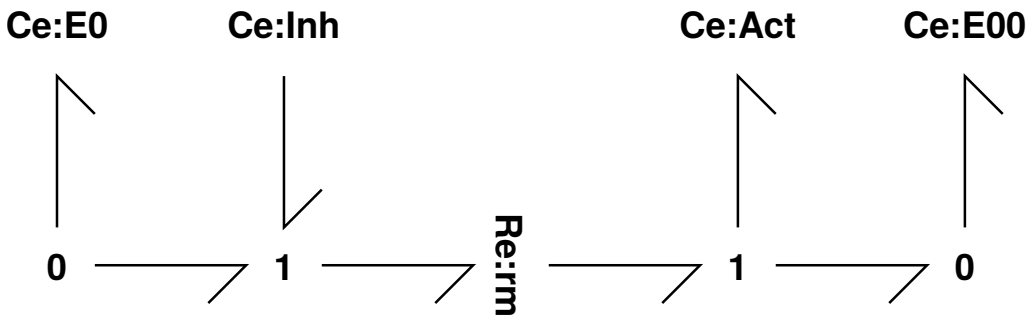
$$\mathrm{C} \overset{r_2}{\rightleftharpoons} \mathrm{B} + \mathrm{E} \tag{2}$$

2.2 Modulation

Competitive inhibition and activation are discussed in chapter 6 of (Cornish-Bowden, 2013).

```
In [4]: ## Modulation
        sbg.model('Mod_abg.svg')
        import Mod_abg
        disp.SVG('Mod_abg.svg')
```

Out[4]:




```
In [5]: s = st.stoich(Mod_abg.model(),quiet=quiet)
        disp.Latex(st.sprintrl(s,chemformula=True))
```

Out[5]:

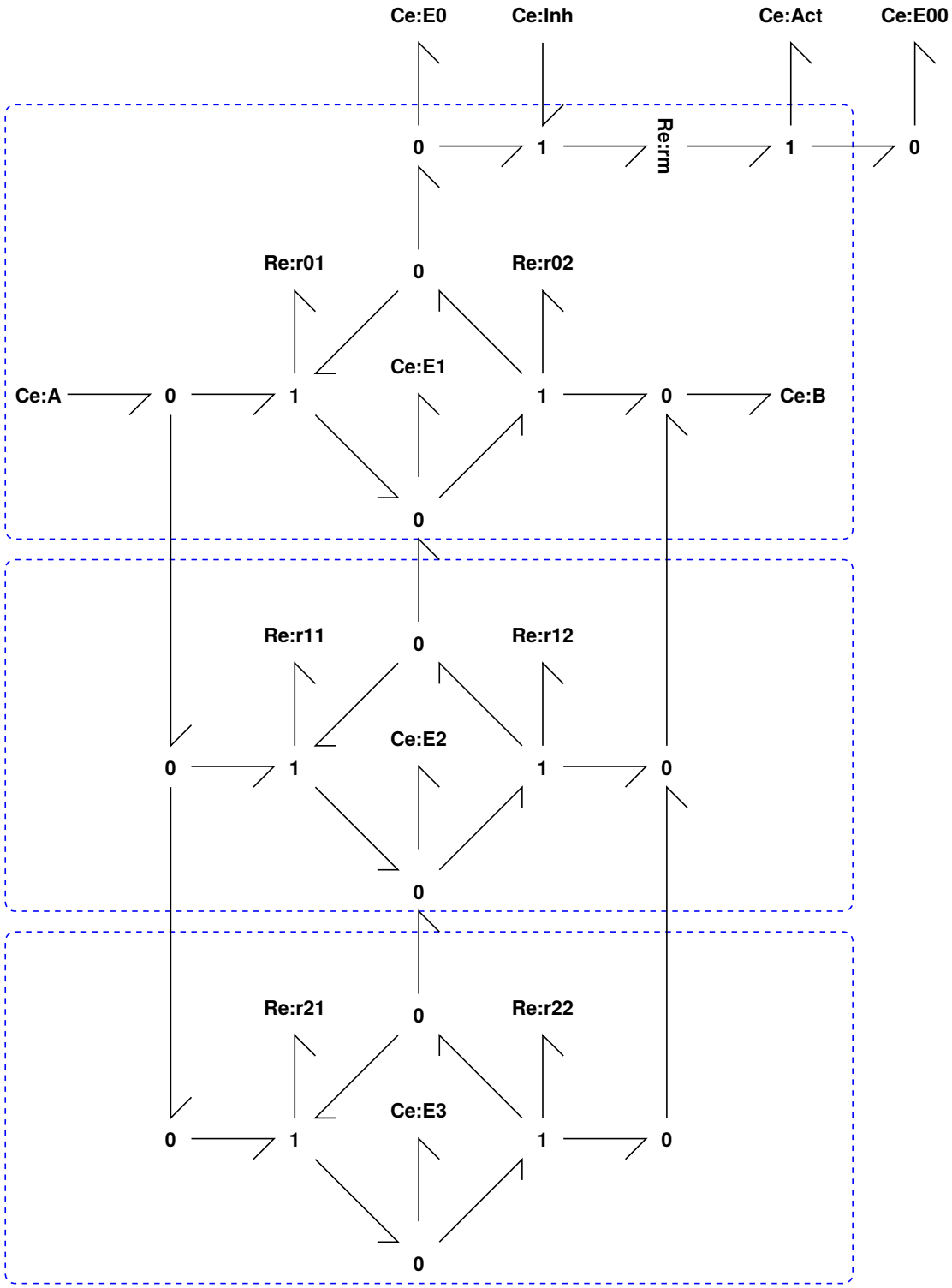
$$E_0 + Inh \overset{rm}{\rightleftharpoons} Act + E_{00} \tag{3}$$

2.3 Two-stage cooperative enzyme-catalysed reaction (N=2) with modulation

The cooperative enzyme-catalysed reaction is modulated by the activation species (Act) and the inhibition species (Inh).

```
In [6]: ## Two-stage cooperative enzyme-catalysed reaction (N=2)
        sbg.model('mCoop_abg.svg',quiet=quiet)
        import mCoop_abg
        disp.SVG('mCoop_abg.svg')
```

Out[6]:




```
In [7]: s = st.stoich(mCoop_abg.model(),quiet=quiet)
        sc = st.statify(s,chemostats=['A','B','Act','Inh'])
        disp.Latex(st.sprintrl(s,chemformula=True))
```

Out[7]:

$$A + E_0 \rightleftarrows^{r_{01}} E_1 \tag{4}$$

$$E_1 \rightleftarrows^{r_{02}} B + E_0 \tag{5}$$

$$A + E_1 \rightleftarrows^{r_{11}} E_2 \tag{6}$$

$$E_2 \rightleftarrows^{r_{12}} B + E_1 \tag{7}$$

$$A + E_2 \rightleftarrows^{r_{21}} E_3 \tag{8}$$

$$E_3 \rightleftarrows^{r_{22}} B + E_2 \tag{9}$$

$$E_0 + Inh \rightleftarrows^{rm} Act + E_{00} \tag{10}$$

2.4 Create cooperative enzyme-catalysed reaction of any degree N

The following code builds an N-stage cooperative enzyme-catalysed reaction using bond-graph tools.

1. N+1 instances of the basic enzyme-catalysed reaction are created and the enzyme and complex renamed.
2. The substrate A, product B and enzymes E1-EN are unified.

In [8]:
```
## Create cooperative enzyme-catalysed reaction of any degree N
## Optionally append a simple reaction
## Optionally use feedback inhibition
def makeCoop(N=3,quiet=True):
    Coop = bgt.new(name='Coop')
    Mod = Mod_abg.model()
    Coop.add(Mod)
    for i in range(N+1):
        RE = RE_abg.model()
        RE.name = 'RE'+str(i)
        mbg.rename(RE,{
                    'E':'E'+str(i),
                    'C':'E'+str(i+1),
                    'r1':'r'+str(i)+'1',
                    'r2':'r'+str(i)+'2'
                    },
                   quiet=quiet)
        Coop.add(RE)

    ## Unify common components
    unified = ['A','B','E0']
    for i in range(N):
        Ei = 'E'+str(i+1)
        unified.append(Ei)
    #print('unified =',unified)
    mbg.unify(Coop,unified,quiet=quiet)
```

```
    ## Stoichiometry
    chemostats = ['A','B','Act','Inh']
    s = st.stoich(Coop,quiet=quiet)
    sc = st.statify(s,chemostats=chemostats)
    if not quiet:
        print(st.sprint(sc,'species'))
        print(st.sprint(sc,'reaction'))
    return s,sc,Coop
```

2.4.1 Generate equations for $N = 2$

Note that these equations are identical to those of the explicit bondgraph.

```
In [9]: ### Generate equations for N=2
        s,sc,Coop = makeCoop(N=2,quiet=quiet)
        disp.Latex(st.sprintrl(s,chemformula=True))
```

Out[9]:

$$\mathrm{Inh} + \mathrm{E_0} \underset{}{\overset{\mathrm{rm}}{\rightleftharpoons}} \mathrm{Act} + \mathrm{E_{00}} \tag{11}$$

$$\mathrm{A} + \mathrm{E_0} \underset{}{\overset{\mathrm{r_{01}}}{\rightleftharpoons}} \mathrm{E_1} \tag{12}$$

$$\mathrm{E_1} \underset{}{\overset{\mathrm{r_{02}}}{\rightleftharpoons}} \mathrm{B} + \mathrm{E_0} \tag{13}$$

$$\mathrm{A} + \mathrm{E_1} \underset{}{\overset{\mathrm{r_{11}}}{\rightleftharpoons}} \mathrm{E_2} \tag{14}$$

$$\mathrm{E_2} \underset{}{\overset{\mathrm{r_{12}}}{\rightleftharpoons}} \mathrm{B} + \mathrm{E_1} \tag{15}$$

$$\mathrm{A} + \mathrm{E_2} \underset{}{\overset{\mathrm{r_{21}}}{\rightleftharpoons}} \mathrm{E_3} \tag{16}$$

$$\mathrm{E_3} \underset{}{\overset{\mathrm{r_{22}}}{\rightleftharpoons}} \mathrm{B} + \mathrm{E_2} \tag{17}$$

2.4.2 Generate pathway equations for $N = 2$

Pathways are generated using the approach of (Gawthrop and Crampin, 2017).

```
In [10]: sp = st.path(s,sc)
         print(st.sprintp(sc))
         disp.Latex(st.sprintrl(sp))
```

```
3 pathways
0:  + r01 + r02
1:  + r11 + r12
2:  + r21 + r22
```

Out[10]:

$$A \Leftrightarrow B \tag{18}$$
$$A \Leftrightarrow B \tag{19}$$
$$A \Leftrightarrow B \tag{20}$$

3 Simulation of Steady-state properties

The steady state properties are investigated using dynamic simulation where slowly varing exogenous quantities are used to induce quasi-steady-state behaviour. In each case, the variable is at a constant value to start with followed by a slowly increasing ramp. The response after the initial reponse is plotted to remove artefacts due to the initial transient.

All parameters are unity except for $K_B = 10^{-6}$ (to approximate an irreversible reaction) and initial states are chosen so that the total enzyme is $e_0 = 1$.

3.1 Set up some parameters for simulation

In [11]:
```
## Set up some parameters for simulation
def setParameter(s,N,e0,K_B=1e-6,modulate=True):
    ## Set up the non-unit parameters and states

    K_E0 = 1
    K_EN = 1/K_E0
    K_m = K_EN/K_E0
    parameter = {}

    ## Set product constant to a small value
    ## to make the ECR approximately irreversible
    parameter['K_B'] = K_B

    ## Set up enzyme parameters and reaction constants
    parameter['K_E0'] = K_E0
    parameter['K_E'+str(N+1)] = K_EN

    ## Modulation
    parameter['kappa_rm'] = 1e3
    parameter['K_E00'] = 1e-1

    ## States
    ## Set total enzyme to e0
    X0 = np.ones(s['n_X'])
    if modulate:
        X0[s['spec_index']['Act']] = 100
        X0[s['spec_index']['E00']] = (e0/(N+3))
        for i in range(N+2):
            Ei = 'E'+str(i)
```

```
            X0[s['spec_index'][Ei]] = (e0/(N+3))
        else:
            for i in range(N+2):
                Ei = 'E'+str(i)
                X0[s['spec_index'][Ei]] = (e0/(N+2))



    return parameter,X0,K_EN,K_m
```

3.2 Simulation code

The flow v is a dynamical function of substrate x_A, activation x_{Act}, inhibition x_{Inh} and cooperativity index N. An approximate steady-state is acjieved by varying one of the three concentrations slowly whilst fixing the other two. The following function does this by declaring the varying function species by the string sX, a fixed species with a number of discrete values as sX1 with values XX1 and the other species as sX2 with value X2. N can take on a range of values.

deriv=True gives a plot of the derivative of the flow with respect to $\log_{10} X$.

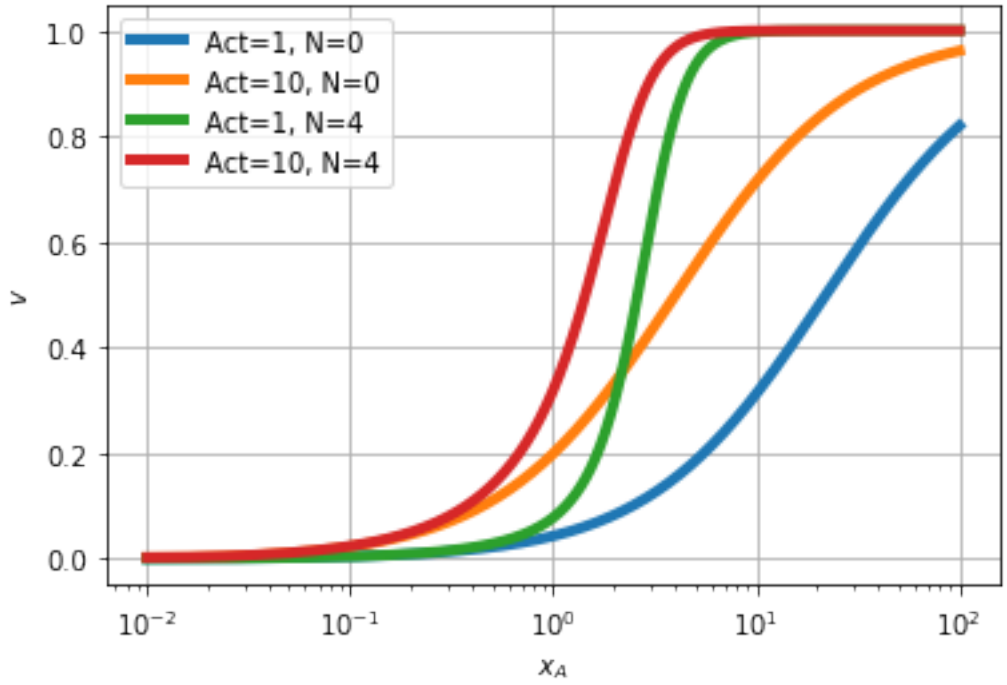
```
In [12]: def label(sX1,sX2,X1,X2,N):
             if N<0:
                 return f'{sX1}={X1}, N={-N} (graphical)'
             else:
                 return f'{sX1}={X1}, N={N}'

         def VaryX(sX='A',sX1='Act',sX2='Inh',XX1=[0.1,1,10],X2=1,NN=[2],K_B=1e-6,deriv=False):

             ## Time
             t_max = int(1e4)
             t = np.linspace(0,t_max,100000)
             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


             ## Set up the chemostats: vary X
             x_max = 1e2
             x_min = 1e-2
             chemo = '{3} + ({0}-{3})*np.heaviside(t-{1},1)*((t-{1})/{2})'.format(x_max,t_0,t_1,
             X_chemo =  {sX:chemo}

             for N in NN:
                 for X1 in XX1:

                     if N<0:
                         ## Use graphical version
```

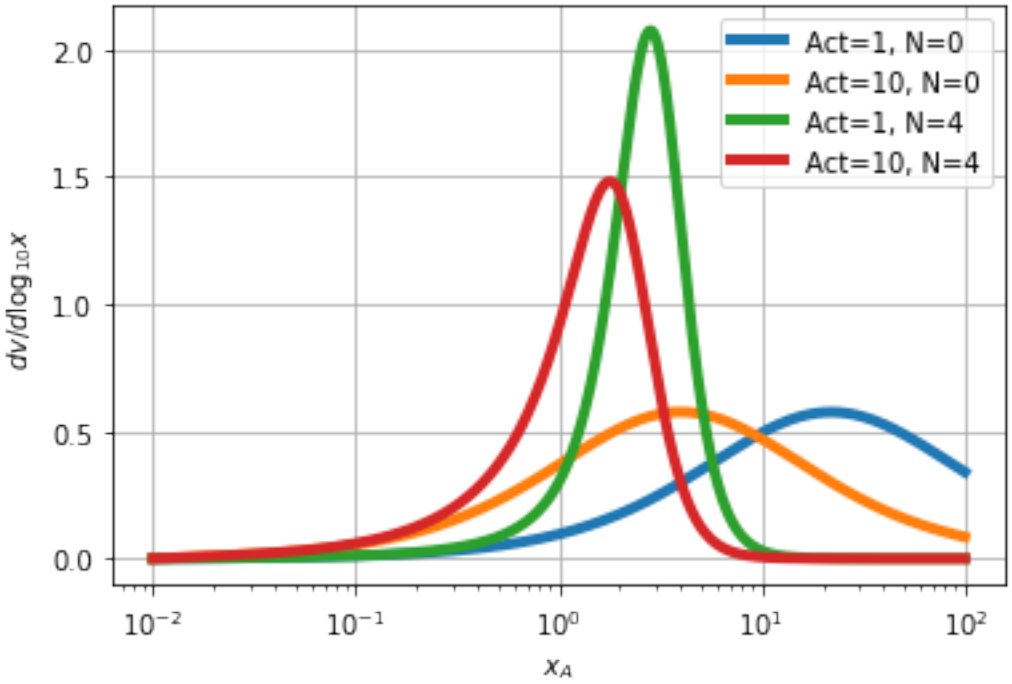
```
            s = st.stoich(mCoop_abg.model(),quiet=quiet)
            sc = st.statify(s,chemostats=['A','B','Act','Inh'])
            lw = 6
            ls = 'dashed'
        else:
            ## Use computational version
            s,sc,Coop = makeCoop(N=N,quiet=quiet)
            lw = 4
            ls = None

        ## Non-unit parameters and states
        e0 = 1 # Total enzyme
        parameter,X0,K_EN,K_m = setParameter(s,abs(N),e0,K_B=K_B)
        X0[s['spec_index'][sX1]] = X1
        X0[s['spec_index'][sX2]] = X2
        dat = st.sim(s,sc=sc,t=t,parameter=parameter,X0=X0,X_chemo=X_chemo,quiet=qu
        dX  = s['N']@(dat['V'].T)
        dX_B = dX[s['spec_index']['B'],:]
        V = dX_B
        X = dat['X'][:,s['spec_index'][sX]]

        if deriv:
            slope = np.gradient(V[-i_1:],np.log10(X[-i_1:]))
            plt.semilogx(X[-i_1:],slope,lw=lw,label=label(sX1,sX2,X1,X2,N),linestyl
            ylabel = '$dv/d \log_{10}{x}$'

        else:
            plt.semilogx(X[-i_1:],V[-i_1:],lw=lw,label=label(sX1,sX2,X1,X2,N),lines
            ylabel = '$v$'



    plt.xlabel('$x_{'+sX+'}$')
    plt.ylabel(ylabel)
    plt.legend()
    plt.grid()
    #plt.title('N = '+str(N))
    plt.show()
```

3.3 Vary the substrate concentration.

The substrate concentration x_A is varied for two values of activation x_{Act} and two values of N. The derivative is also plotted.

```
In [13]: NN = [0,4]
         VaryX(sX='A',sX1='Act',sX2='Inh',XX1=[1,10],X2=1,NN=NN)
         VaryX(sX='A',sX1='Act',sX2='Inh',XX1=[1,10],X2=1,NN=NN,deriv=True)
```

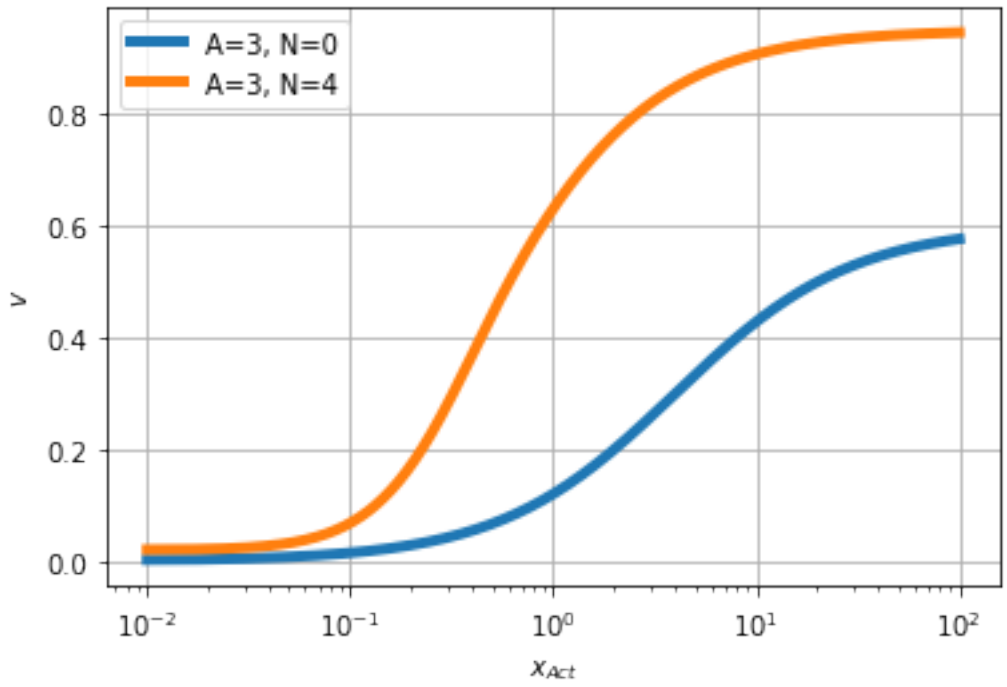

Act=1, N=0
Act=10, N=0
Act=1, N=4
Act=10, N=4
1.0
0.8
0.6
0.4
0.2
0.0
v
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
x_A

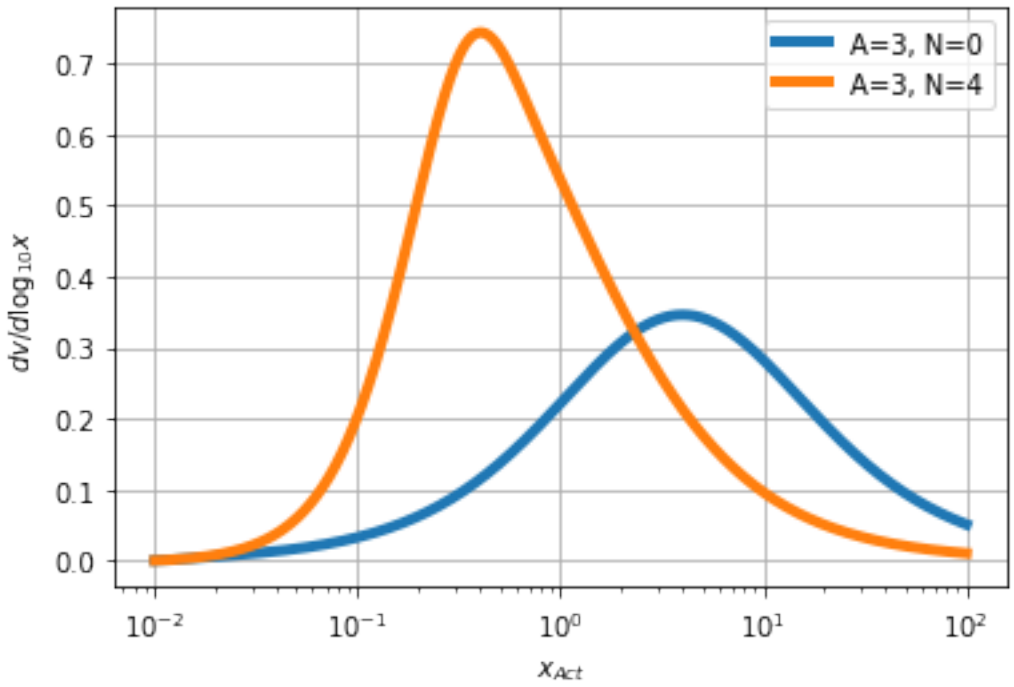

Act=1, N=0
Act=10, N=0
Act=1, N=4
Act=10, N=4
2.0
1.5
1.0
0.5
0.0
$dv/d\log_{10}x$
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
x_A

3.4 Vary the activation species concentration.

The activation species concentration x_{Act} is varied for two values of N. The derivative is also plotted.

```
In [14]: VaryX(sX='Act',sX1='A',sX2='Inh',XX1=[3],X2=1,NN=NN,deriv=False)
         VaryX(sX='Act',sX1='A',sX2='Inh',XX1=[3],X2=1,NN=NN,deriv=True)
```

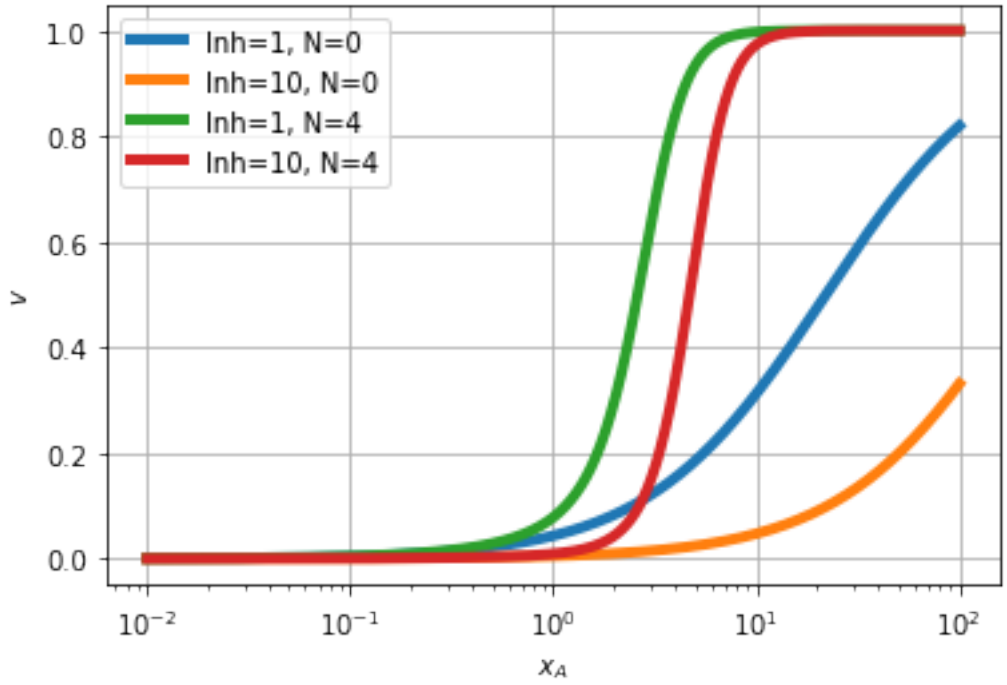


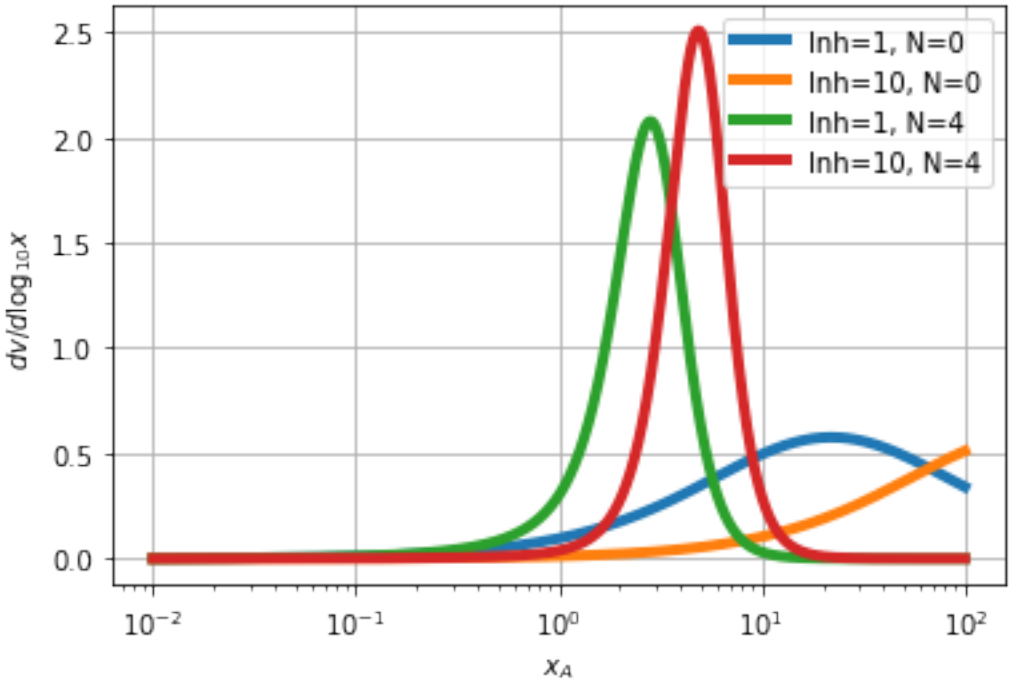


3.5 Vary the substrate concentration.

The substrate concentration x_A is varied for two values of inhibition x_{Inh} and two values of N. The derivative is also plotted.

```
In [15]: VaryX(sX='A',sX1='Inh',sX2='Act',XX1=[1,10],X2=1,NN=NN)
         VaryX(sX='A',sX1='Inh',sX2='Act',XX1=[1,10],X2=1,NN=NN,deriv=True)
```

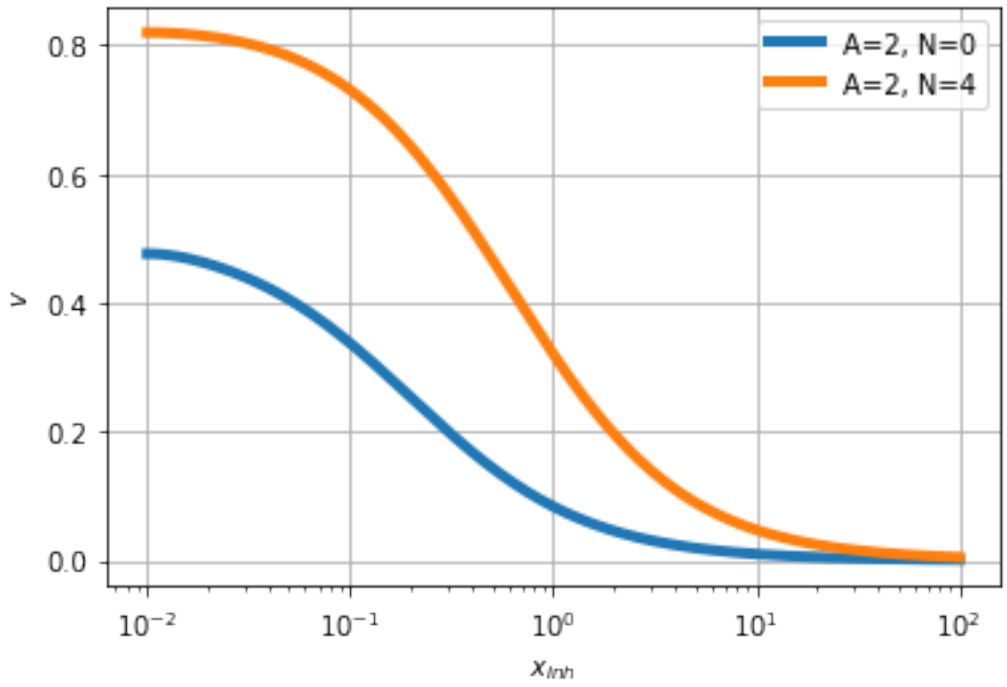

Inh=1, N=0
Inh=10, N=0
Inh=1, N=4
Inh=10, N=4
v
x_A

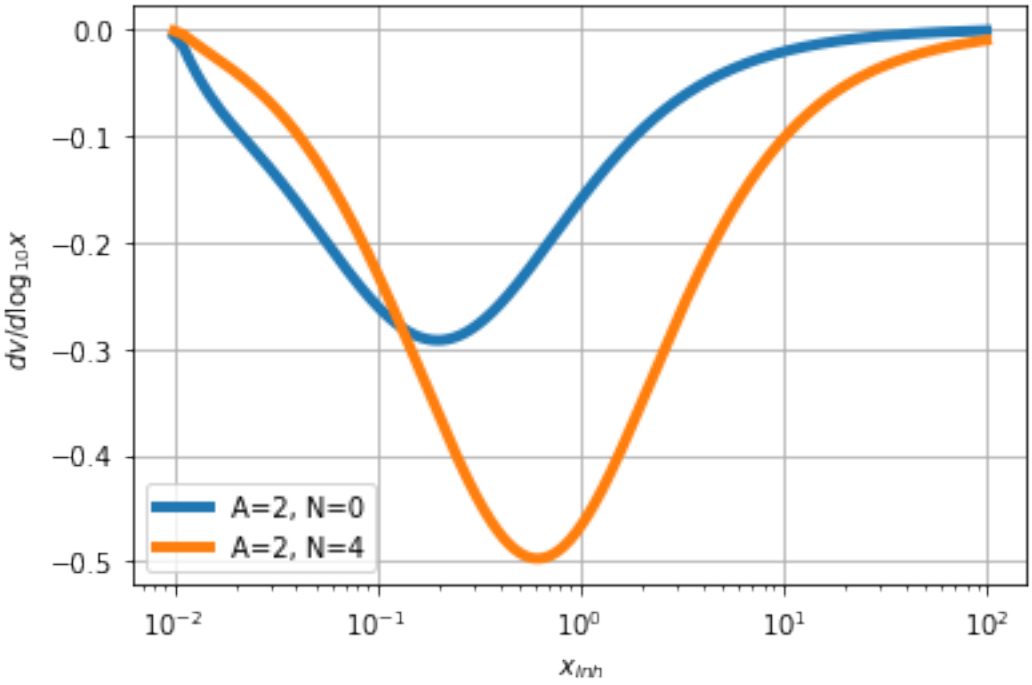

Inh=1, N=0
Inh=10, N=0
Inh=1, N=4
Inh=10, N=4
$dv/d\log_{10}x$
x_A

3.6 Vary the inhibition species concentration.

The inhibition species concentration x_{Inh} is varied for two values of N. The derivative is also plotted.

```
In [16]: VaryX(sX='Inh',sX1='A',sX2='Act',XX1=[2],X2=1,NN=NN)
         VaryX(sX='Inh',sX1='A',sX2='Act',XX1=[2],X2=1,NN=NN,deriv=True)
```

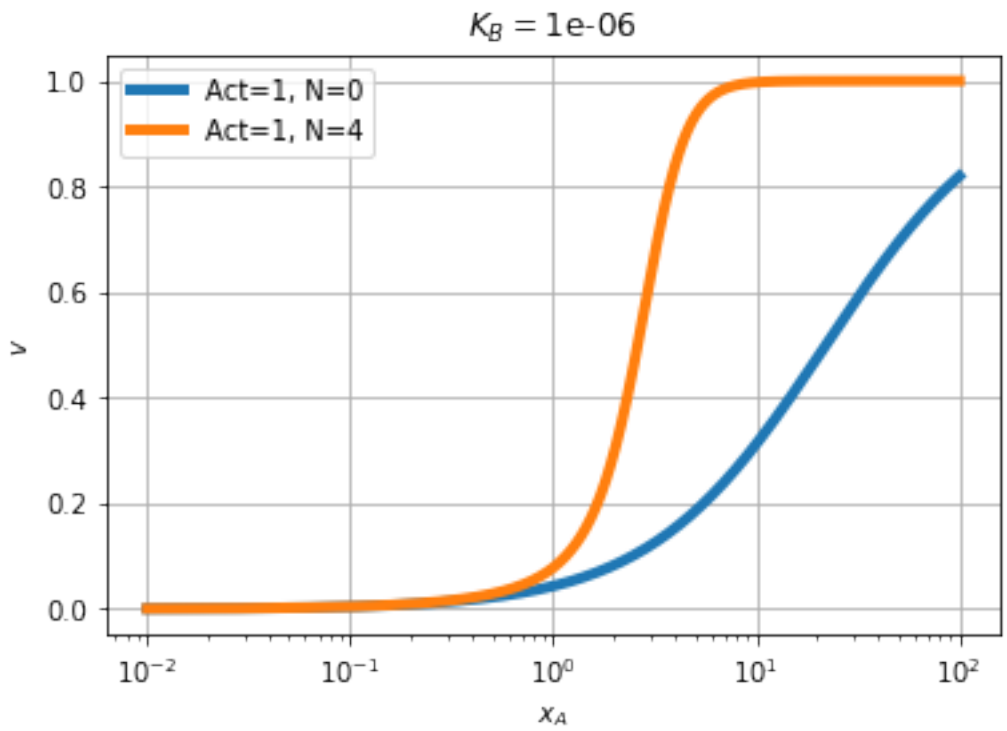


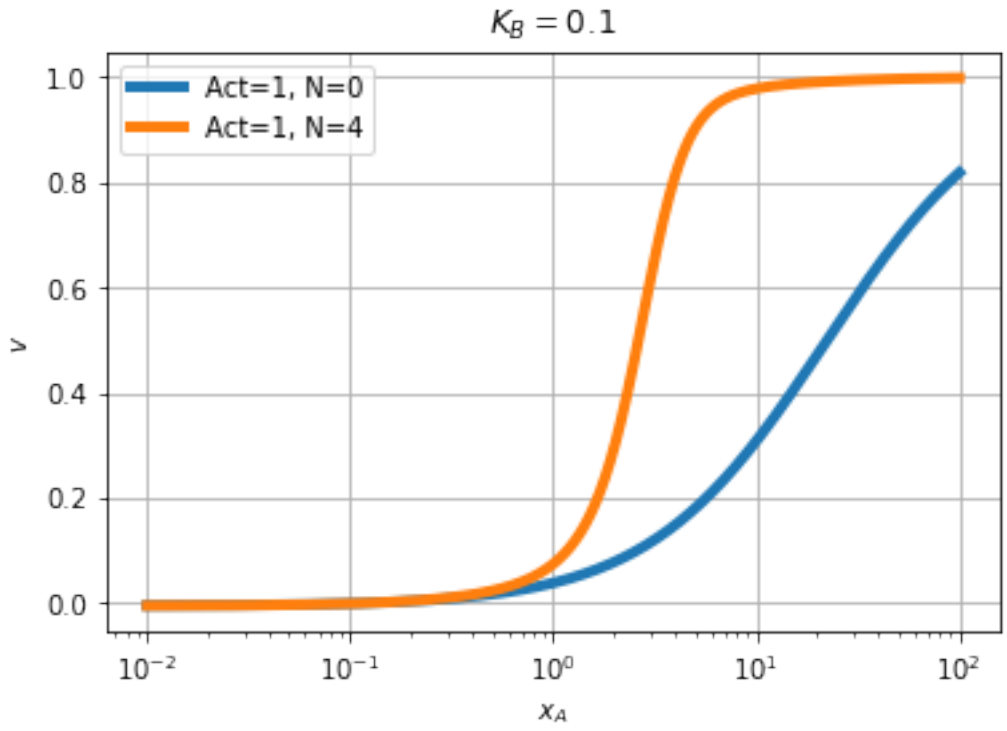


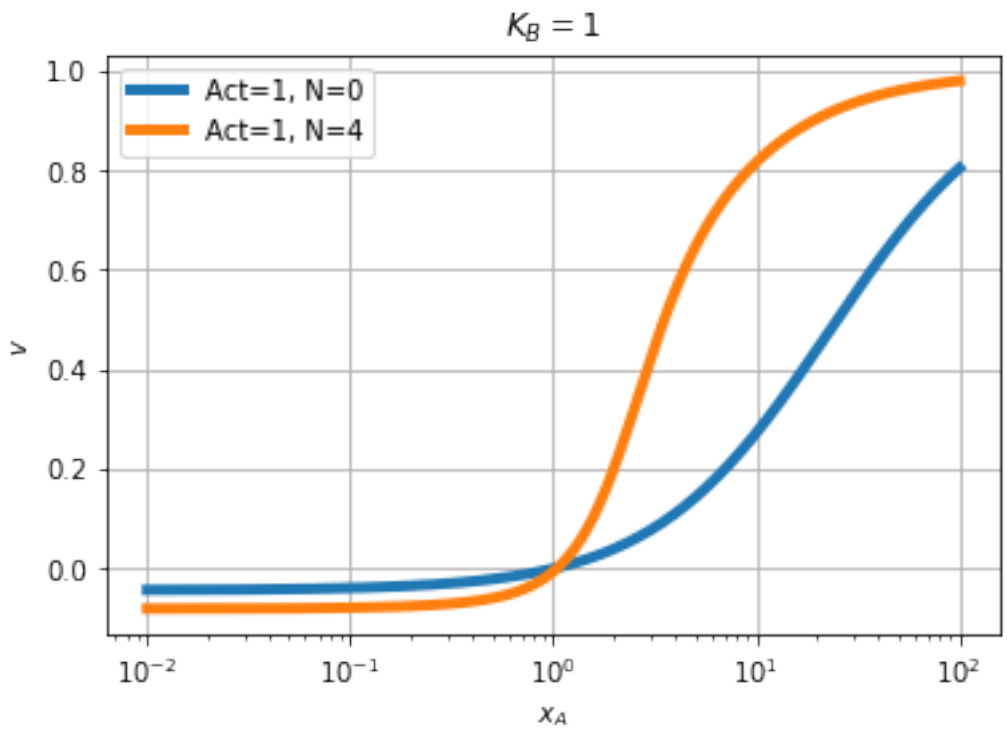
3.7 Effect of product

The above simulations have $K_B = 10^{-6}$; the following shows the effect of increasing K_B.

```
In [17]: for K_B in [1e-6,0.1,1]:
             plt.title('$K_B = $'+str(K_B))
             VaryX(sX='A',sX1='Act',sX2='Inh',XX1=[1],X2=1,NN=NN,K_B=K_B)
```


$K_B = 1e\text{-}06$
Act=1, N=0
Act=1, N=4
1.0
0.8
0.6
0.4
0.2
0.0
v
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
x_A

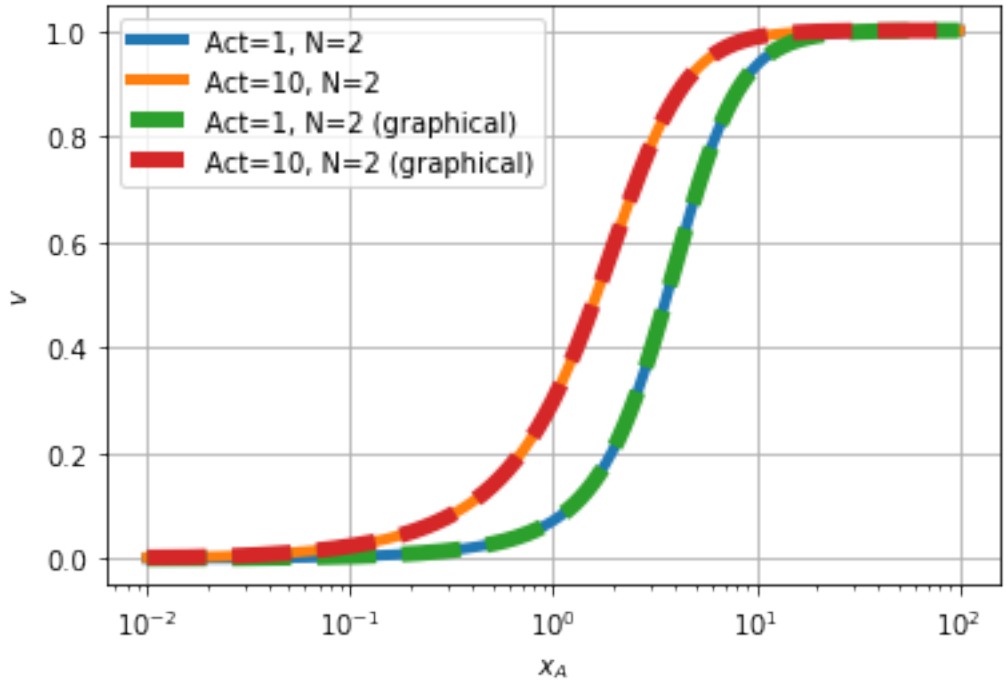

$K_B = 0.1$
Act=1, N=0
Act=1, N=4
1.0
0.8
0.6
0.4
0.2
0.0
v
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
x_A

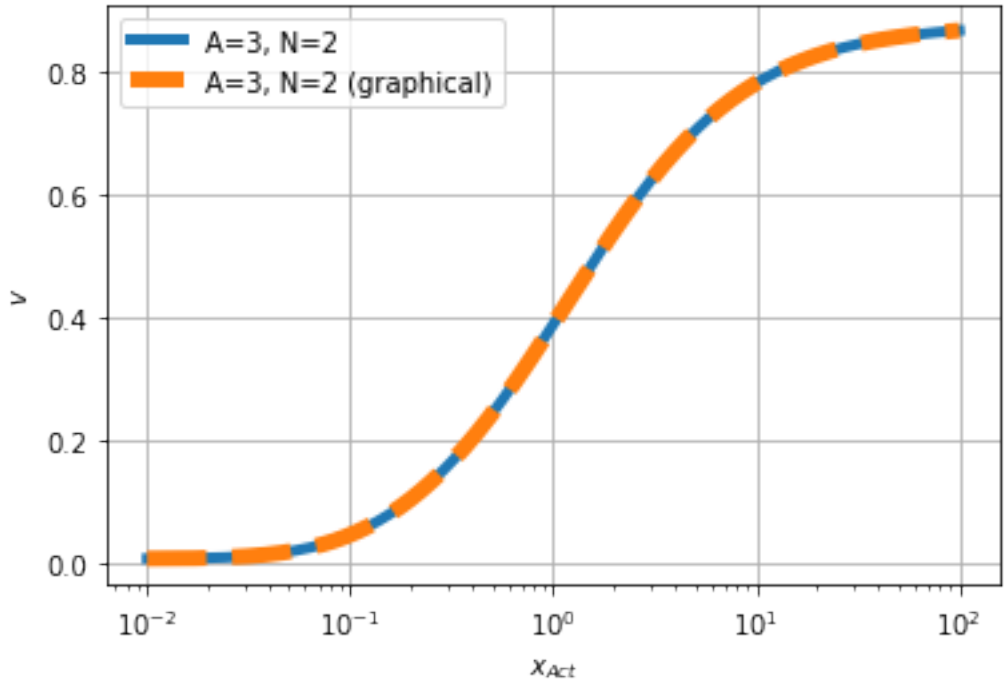


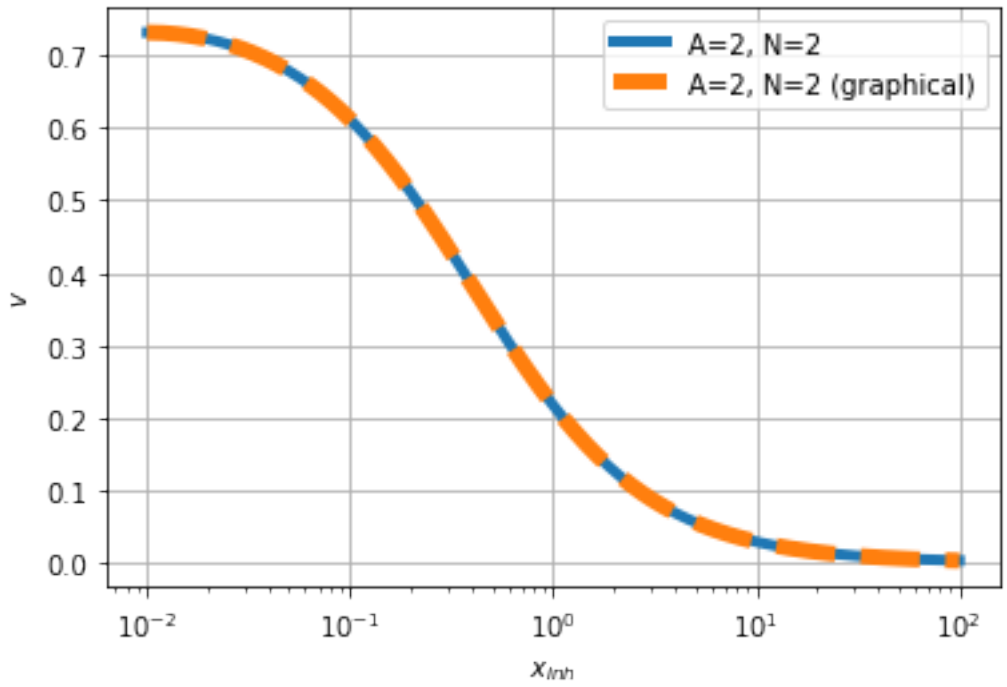
3.8 Compare graphical and computational

The graphical bond-graph representation corresponds to N=2 (activated in the code by setting N=-2). This section checks that the simulation gives the same results for the coresponding computational form of the bond graph (N=2).

```
In [18]: NN = [2,-2]
         VaryX(sX='A',sX1='Act',sX2='Inh',XX1=[1,10],X2=1,NN=NN)
         VaryX(sX='Act',sX1='A',sX2='Inh',XX1=[3],X2=1,NN=NN)
         VaryX(sX='Inh',sX1='A',sX2='Act',XX1=[2],X2=1,NN=NN)
```


Act=1, N=2
Act=10, N=2
Act=1, N=2 (graphical)
Act=10, N=2 (graphical)
1.0
0.8
0.6
0.4
0.2
0.0
v
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
x_A


A=3, N=2
A=3, N=2 (graphical)
0.8
0.6
0.4
0.2
0.0
v
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
x_{Act}



4 Discussion

- The maximum flowrate is unchanged by activation or inhibition.
- Increasing the cooperativity order N increases the slope of the curves and the incremental gain with respect to substrate, activation and inhibition.
- It is necessary that the product potential is small. It is a chemostat here and this is acjeived by a small K_B. In a real situation, this could be acheived by removing product rapidly, having a product with small standard potential or using energy pumping via, for example ATP hydrolysis.
- the behaviour is dependent on the parameters of the particular enzyme-catalysed reaction; those used here are for illustration.

References

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