Allosteric inhibition as a control actuator - modular approach: passive and active feedback

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Contents

Note: this is the modularAllosteric.ipynb notebook. The PDF version "Allosteric inhibition as a control actuator - modular approach: passive and active feedback" is available here.

1 Introduction

Using the methods of control theory to examine and reexamine the behaviour of living systems is well-established (Craik, 1947) (Wiener, 1961) (Bayliss, 1966) (Savageau, 2009) (Jagacinski and Flach, 2003) (Iglesias and Ingalls, 2010) (Wellstead et al., 2008) (Drion et al., 2015) (Del Vecchio, 2013). This notebook examines the enzyme-catalysed reaction as a control actuator and its behavior within a feedback loop controlling product concentration. The feedback mechanism used is *Allosteric inhibition* - see section 1.4.3 (Keener and Sneyd, 2009).

- The notebook fECR looks at an alternative feedback mechanism : competitive inhibition.
- As discussed in the notebook Allosteric, competitive inhibition is a special case of allosteric inhibition.
- As discussed in the notebook fECR, the control methodology is based on Linearisation.
- This notebook introduces a novel method based on *chemostats* for deducing the *feedback loop-gain* $L(s)$ from the bond graph describing the biomolecular system. $L(s)$ is a crucial component of feedback control analysis.
- The presence of *active* feedback via allosteric inhibition and *passive* feedback via the admittance of the reaction chain at the product is noted. The passive feedback has a stabilising effect.

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 sym
        import matplotlib.pyplot as plt
        import IPython.display as disp
        import copy

        ## Stoichiometric analysis
        import stoich as st

        ## SVG bg representation conversion
        import svgBondGraph as sbg

        ## Modularity
        import modularBondGraph as mbg

        ## Control systems package
        import control as con

        ## Stoichiometry to BG
```

```
import stoichBondGraph as stbg

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

## Set slycot=True if slycot is installed (see control module)
slycot=True

## For reimporting: use imp.reload(module)
import importlib as imp

## Printing options
np.set_printoptions(precision=3)
fmt = '{:5.3f}'

## Allow output from within functions
from IPython.core.interactiveshell import InteractiveShell
InteractiveShell.ast_node_interactivity = "all"

## Minreal (minimum realisation algorithm) tolerance
tol = 1e-2

## Chemostat EM and CM
chemostatEMCM = False

## Use fixed-length reaction chain (N_reac=3)
## Fixed = False allows arbitary length reaction chains using N_reac = int.
Fixed = True
```

1.2 Derive stoichiometry from bond graph

```
In [2]: def stoichiometry(abg,chemostats=[]):
            s = st.stoich(abg,quiet=quiet)
            sc = st.statify(s,chemostats=chemostats)
            return s,sc
```

2 Enzme catalysed reaction with allosteric inhibition

2.1 Bond graph model

- **Ce:A**: substrate
- **Ce:B**: product
- **Ce:B0**: product sink
- **Ce:E**: enzyme
- **Ce:C**: enzyme bound to A
- **Ce:F** and **Ce:G**: species pumping the reaction
- **Ce:B**: product
- **Ce:EM**: enzyme bound to M

- **Ce:CM**: complex C bound to M
- Corresponding reactions are:

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

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

$$\mathrm{B} \overset{r_3}{\rightleftharpoons} \mathrm{B}_0 \tag{3}$$

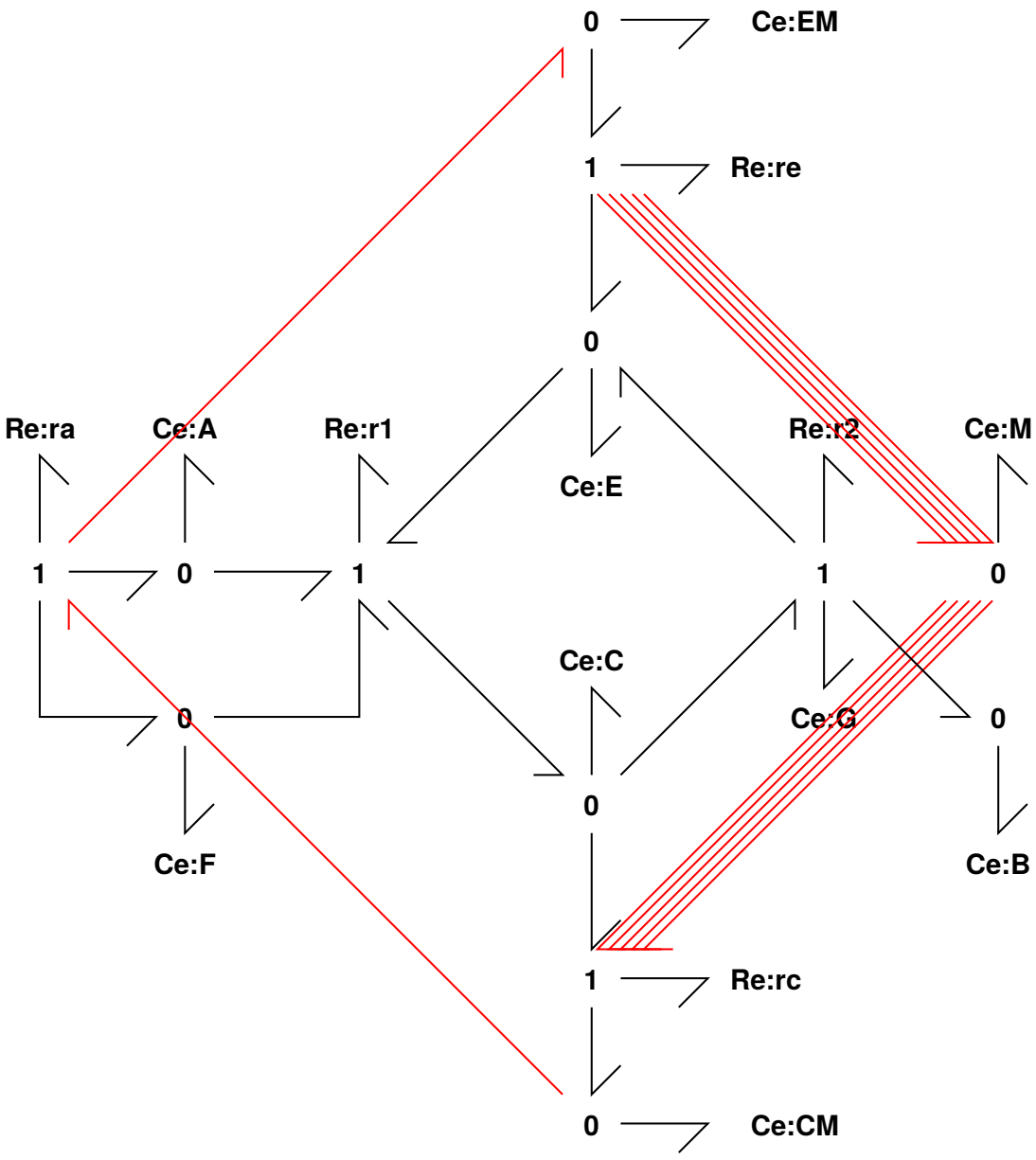
$$\mathrm{CM} \overset{ra}{\rightleftharpoons} \mathrm{A} + \mathrm{EM} \tag{4}$$

$$5\,\mathrm{B} + \mathrm{C} \overset{rc}{\rightleftharpoons} \mathrm{CM} \tag{5}$$

$$\mathrm{EM} \overset{re}{\rightleftharpoons} 5\,\mathrm{B} + \mathrm{E} \tag{6}$$

```
In [3]: sbg.model('aiRE_abg.svg',quiet=quiet)
        import aiRE_abg
        #abg = aiRE_abg
        disp.SVG('aiRE_abg.svg')
```

Out[3]:




```
In [4]: ## Stoichiometry
        chemostats=['A','M','F','G']
        if chemostatEMCM:
            chemostats += ['EM','CM']

        s,sc = stoichiometry(aiRE_abg.model(),chemostats=chemostats)

        print('Reactions:')
        disp.Latex(st.sprintrl(s,chemformula=True))
        sp = st.path(s,sc)
```

```
Reactions:
```

Out[4]:

$$A + E + F \overset{r_1}{\rightleftharpoons} C \tag{7}$$
$$C \overset{r_2}{\rightleftharpoons} B + E + G \tag{8}$$
$$CM \overset{ra}{\rightleftharpoons} A + EM + F \tag{9}$$
$$C + 5M \overset{rc}{\rightleftharpoons} CM \tag{10}$$
$$EM \overset{re}{\rightleftharpoons} E + 5M \tag{11}$$

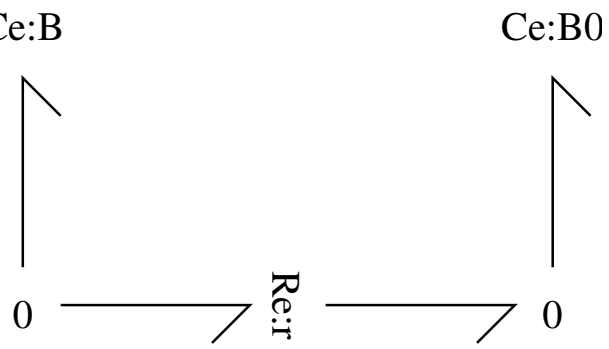
2.2 Controlled system bond graph

2.2.1 Arbitrary length N_{reac}

This corresponds to the arbitary length chain when Fixed=False It is the same as the fixed length chain when N_reac=3.

In [5]:
```
sbg.model('BB_abg.svg',quiet=quiet)
import BB_abg
disp.SVG('BB_abg.svg')
```

Out[5]:



In [6]:
```
def CLBG(aiRE_abg,abg,N=1):

    if N is None:
        NBB = abg.model()
    else:
        ## Create chain
        NBB = mbg.chain(abg.model(),inport='B',outport='B0',N=N,quiet=quiet)
        if N>1:
            ## Rename penultimate state
```

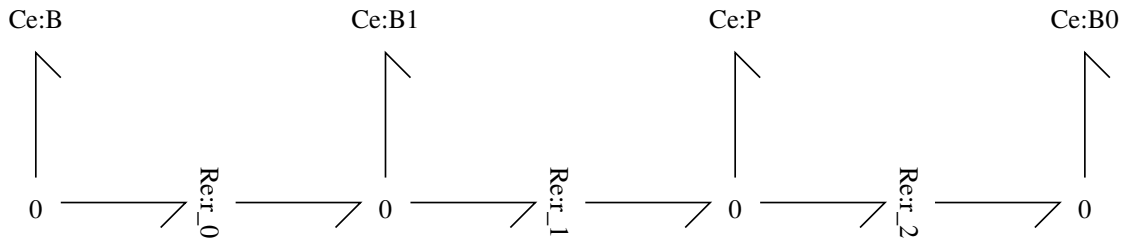
```
            ISN1 = 'IS'+str(N-1)
            mbg.rename(NBB,{ISN1:'P'})
        else:
            mbg.rename(NBB,{'B':'P'})

    ## Create composite system
    aiRE = aiRE_abg.model()
    if N is 1:
        mbg.rename(aiRE,{'B':'P'})

    mbg.rename(aiRE,{'M':'P'})

    Allosteric = bgt.new(name='Allosteric')
    Allosteric.add(aiRE,NBB)
    if N is 1:
        common = ['P']
    else:
        common = ['B','P']


    mbg.unify(Allosteric,common=common,quiet=quiet)

    return Allosteric
```

2.2.2 Fixed length $N_{reac} = 3$

This corresponds to the fixed-length chain when Fixed=True

```
In [7]: sbg.model('ReacChain_abg.svg',quiet=quiet)
        import ReacChain_abg
        disp.SVG('ReacChain_abg.svg')
```

Out[7]:



2.3 Stoichiometry and reactions

```
In [8]: if Fixed:
            abg = ReacChain_abg
```

```
        N_reac = None
    else:
        abg = BB_abg
        ## Number of reactions in the chain
        N_reac = 3


    ## Create composite closed-loop system
    Allosteric = CLBG(aiRE_abg,abg,N=N_reac)

    ## Stoichiometry
    chemostats=['A','BO','F','G']
    if chemostatEMCM:
        chemostats += ['EM','CM']
    s,sc = stoichiometry(Allosteric,chemostats=chemostats)

    print('Reactions:')
    disp.Latex(st.sprintrl(s,chemformula=True))
    sp = st.path(s,sc)
    print('Pathway reactions:')
    print(st.sprintp(sc))
    disp.Latex(st.sprintrl(sp,chemformula=False))
```

```
Renaming components within aiRE
        Renaming M to P
Reactions:
```

Out[8]:

$$\mathrm{A} + \mathrm{E} + \mathrm{F} \overset{r_1}{\rightleftharpoons} \mathrm{C} \tag{12}$$

$$\mathrm{C} \overset{r_2}{\rightleftharpoons} \mathrm{E} + \mathrm{G} + \mathrm{B} \tag{13}$$

$$\mathrm{CM} \overset{ra}{\rightleftharpoons} \mathrm{A} + \mathrm{EM} + \mathrm{F} \tag{14}$$

$$\mathrm{C} + 5\,\mathrm{P} \overset{rc}{\rightleftharpoons} \mathrm{CM} \tag{15}$$

$$\mathrm{EM} \overset{re}{\rightleftharpoons} \mathrm{E} + 5\,\mathrm{P} \tag{16}$$

$$\mathrm{B} \overset{r_0}{\rightleftharpoons} \mathrm{B}_1 \tag{17}$$

$$\mathrm{B}_1 \overset{r_1}{\rightleftharpoons} \mathrm{P} \tag{18}$$

$$\mathrm{P} \overset{r_2}{\rightleftharpoons} \mathrm{B}_0 \tag{19}$$

```
Pathway reactions:
2 pathways
0:  + r1 + ra + rc + re
1:  + r1 + r2 + r_0 + r_1 + r_2
```

Out[8]:

$$\Leftrightarrow \tag{20}$$

$$A + F \Leftrightarrow G + B0 \tag{21}$$

2.4 Utility functions

In [9]:
```
def plotStep(tf,chemo,spec,T):

    t,y = con.step_response(tf,T=T)
    plt.plot(t,y)
    plt.grid()
    plt.xlabel('t')
    plt.ylabel('x_B')
    plt.title('Step response from '+chemo+' to '+spec)
    plt.show()

def showTF(tf):
    ## Show info
    print(tf)
    in_gain_X = con.dcgain(tf)
    print('\tgain:',fmt.format(in_gain_X))
    print('\tpoles:', con.pole(tf))
    print('\tzeros:', con.zero(tf))
    print('\n')

def extractTF(TF,chemo,spec):

    ## Index of product
    species = s['species']
    i_prod = species.index(spec)

    ## Index of input
    i = chemostats.index(chemo)

    ## Extract tf
    #print('\nTransfer function from','x_'+chemo,'to','x_'+spec)
    tf = con.minreal(TF[i_prod,i],tol=tol,verbose=True)

    #showTF(tf)

    return tf

def extractTFflow(TF,chemo,chemostats,reac):

    ## Index of reaction
    reaction = s['reaction']
```

```
    i_v = reaction.index(reac)

    ## Index of input
    i = chemostats.index(chemo)

    ## Extract tf
    #print('\nTransfer function from','x_'+chemo,'to','v_'+reac)
    tf = con.minreal(TF[i_v,i],tol=tol,verbose=True)

    #showTF(tf)

    return tf
```

3 Closed-loop analysis

The function **ClosedLoop** derives the closed-loop properteis of the system from the bond graph model in the following steps:

- Extract stoichiometry
- Simulate the system to give a steady state
- linearise the closed-loop system about this steady state to give:
- the multivariable transfer function relating chemostats to species states
- the multivariable transfer function relating chemostats to reaction flows
- extract the scalar transfer functions relating the 'disturbance' x_{B0} to
  - the product state x_P
  - the 'actuator' flow v_{r2}

```
In [10]: def ClosedLoop(abg,chemostats,parameter,X0,t_ss,t_step,quiet=False):
             ## Analyse the system

             cabg = copy.copy(abg)

             ## Stoichiometry
             s,sc = stoichiometry(cabg,chemostats=chemostats)

             print('Closed-loop. Order = ',sc['n_x'])

             ## Steady-state simulation
             t = np.linspace(0,t_ss,1000)
             ssdat = st.sim(s,sc=sc,t=t,parameter=parameter,X0=X0,quiet=quiet)
             #st.plot(s,ssdat,species=['E','C','EM','CM'],reaction = ['r2'])

             ## Use the final value as the steady-state
             x_ss = ssdat['X'][-1,:]
             v_ss = ssdat['V'][-1,:]

             if not quiet:
```

```
        print(s['species'])
        print('x_ss =', x_ss)
        print(s['reaction'])
        print('v_ss = ', v_ss)

    ##Linearise
    SysX = st.lin(s,sc,x_ss=x_ss,parameter=parameter,outvar='X',quiet=quiet)
    SysV = st.lin(s,sc,x_ss=x_ss,parameter=parameter,outvar='V',quiet=quiet)

    ## Multivariable transfer functions
    TF = con.ss2tf(SysX)
    TFV = con.ss2tf(SysV)

    ## Scalar transfer function to x_M
    tf_B0 = extractTF(TF,'B0','P')
    t,x_B = con.step_response(tf_B0,T=t_step)

    return x_ss,v_ss,tf_B0
```

4 Loop-gain analysis

- The function LoopGain deduces the feedback loop-gain from the (closed-loop) bondgraph.
- The product component **Ce:P** is converted into a chemostat. This 'breaks the loop' and enables the open-loop transfer functions relating x_P to the various flows impinging on the corresponding **0** junction to be computed. In this case the relevant flows are:
  - v_{r1} - the main feedback path
  - v_{r2} - the product flow flow absorbed by **Ce:B0**
  - $5v_{re}$ - the flow transiently absorbed by **Ce:EM**
  - $5v_{ce}$ - the flow transiently absorbed by **Ce:CM**
- As will be seen, the the main feedback path to v_{r1} is the sum of two transfer functions:
  - an *active* path via the allosteric inhibition module
  - a *passive* path via the admittance of the reaction chain at the product **Ce:P**
- The function LoopGain follows:

```
In [11]: def LoopGain(abg,chemostats,parameter,x_ss,quiet=False):
             """Compute the loop gain tf"""

             ## Add the chemostat for B and recompute stoichiometry
             chemostatsL = chemostats+['P']
             sL,scL = stoichiometry(copy.copy(abg),chemostats=chemostatsL)

             print('Open-loop. Order = ',scL['n_x'])
             ## Linearise using the appropriate steady-state x_ss
             SysL = st.lin(sL,scL,x_ss=x_ss,parameter=parameter,outvar='V',quiet=quiet)
```

```
## The multivariable transfer function
TFL = con.ss2tf(SysL)

## Stoichiometry of feedback
N = sL['N'] # Stoichiometric matrix
i_P = sL['species'].index('P')
N_P = N[i_P,:] # Row of N corresponding to B

## Indices of reactions impinging on Ce:
j_FB = np.nonzero(N_P)[0][:]

## Reactions impinging on B and the transfer functions
reaction = sL['reaction']
R = []
FB = {}
fb = 0
for j in j_FB:
    r = reaction[j]
    R.append(r)

    ## Transfer function from product P to reaction r
    FB_r = extractTFflow(TFL,'P',chemostatsL,r)
    FB_r *= -N_P[j]

    ## Extract active and passive components of main feedback TF
    if r in [r_last_1]:
        #print('Doing',r_last_1)
        num = FB_r.num[0][0]
        #print('num',num)
        den = FB_r.den[0][0]
        #print('den',den)
        n_num = len(num)
        num_act = [num[n_num-1]]
        FB['fb_act'] = con.tf(num_act,den)
        print('\nActive feedback from P to',r)
        showTF(FB['fb_act'])
        num_pas = copy.copy(num)
        num_pas[n_num-1] = 0
        FB['fb_pas'] = con.tf(num_pas,den)
        print('\nPassive feedback from P to',r)
        showTF(FB['fb_pas'])
    FB[r] = FB_r
    fb = con.parallel(fb,FB[r])
    print('\nFeedback from P to ',r)
    showTF(FB[r])
#print('\nRelevant reactions:', R)

if N_reac is None:
```

```
        r_N1 = 'r_2'
    else:
        r_N1 = 'r_'+str(N_reac-1)
    print('\nGain from B0 to', r_N1)
    FF = -extractTFflow(TFL,'B0',chemostatsL,r_N1)
    showTF(FF)

    ## Total feedback transfer function
    print('\nNet feedback')
    fb = con.minreal(fb,tol=tol,verbose=not quiet)
    showTF(fb)

    ## More tfs into FB
    FB['net'] = fb
    FB['rec'] = con.parallel(FB['re'],FB['rc'])

    ## Transfer function of CE:P
    G = con.tf([1],[1,0])
    print("\nForward gain to P")
    showTF(G)

    ## The loop gains: feedbacks from P to r multiplied by G: TF of P
    L = {}
    for key,tf in FB.items():
        L[key] = con.series(G,FB[key])

    ## Compute the closed-loop TF from the open-loop TF
    print("\nClosed loop")
    CL = con.minreal(con.feedback(G,sys2=FB['net']),tol=tol,verbose=not quiet)
    CL = con.series(CL,FF)
    #showTF(CL)

    return CL,L
```

5 Loop gain analysis of Allosteric feedback

5.1 Utility functions

In [12]:
```
def plotCL(CL,CLL,t_step):

    t_CL,y_CL = con.step_response(CL,T=t_step)
    t_CLL,y_CLL = con.step_response(CLL,T=t_step)
    plt.plot(t_CL,y_CL,label='Direct')
    plt.plot(t_CLL,y_CLL,label='Computed')
    plt.grid()
    plt.xlabel('$t$')
    plt.ylabel('$x_P$')
```

```
        plt.legend()
        plt.show()
        return t_CL,y_CL

    def plotOL(L):

        w = np.logspace(-1,1,200)
        #w = None
        Legend = []
        plt.figure(figsize=(10,10))
        print('Bode diagram: loop gain components')
        for key,tf in L.items():
            if not key in ['re','rc','fb_pas','fb_act']:
                mag,phase,ww = con.bode(tf,w,Plot=True)
                Legend += [key]
        plt.legend(Legend)
        plt.title('Loop gain componenents')
        plt.show()


        Legend = []
        plt.figure(figsize=(10,10))
        print('Bode diagram: Passive and active loop-gain components')
        for key,tf in L.items():
            if key in ['fb_pas','fb_act',r_last_1]:
                mag,phase,ww = con.bode(tf,w,Plot=True)
                Legend += [key]
        plt.legend(Legend)
        plt.show()

        re,im,ww = con.nyquist(L['net'],Plot=False)
        plt.title('Nyquist Diagram')
        plt.plot(re,im)
        plt.axis('equal')
        lim = 2
        plt.xlim(-lim,lim)
        plt.ylim(-lim,lim)
        plt.grid()
        theta = np.linspace(0,2*np.pi)
        plt.plot(np.sin(theta),np.cos(theta))
```

5.2 Numerical parameters

```
In [13]: ## Parameters
         X0_A = 1
         K_A = 1
         K_B = 1
         K_P = 1
```

```
K_C = 1
K_E = 1
K_F = 1000
K_G = 1/K_F

K_EM = 1
K_CM = 1

kappa = 1000
kappa_r1 = kappa
kappa_r2 = kappa

## Closed-loop allosteric inhibition
## Set kappa_ra = kappa_rc = 0 for feedback inhibition
kappa_ra = 1
kappa_rc = 1
kappa_re = 1

K_B0 = K_P
X0_B0 = 1
#kappa_r3 = 1

X0_E = 0.01
X0_C = 0.01

pars = ['K_A','K_B','K_P','K_C','K_E','K_F','K_G',
        'K_EM','K_CM',
        'kappa_r1','kappa_r2','kappa_ra','kappa_rc','kappa_re',
        'K_B0']
parameter = {}
for par in pars:
    parameter[par] = eval(par)

if N_reac is  None:
    r_last = 'r_2'
    r_last_1 = 'r_1'
else:
    r_last = 'r_'+str(N_reac-1)

    if N_reac>1:
        r_last_1 = 'r_'+str(N_reac-2)
        kappa_last_1 = 'kappa_'+r_last_1
        parameter[kappa_last_1] = 1
        print(kappa_last_1,'=', parameter[kappa_last_1])

kappa_last = 'kappa_'+r_last
parameter[kappa_last] = 1
print(kappa_last,'=', parameter[kappa_last])
```

```
        states = ['A','B0','E','C']
        X0 = np.ones(s['n_X'])
        for state in states:
            X0_ = 'X0_'+state
            val = eval(X0_)
            print(X0_,'=',val)
            X0[s['species'].index(state)] = val

        if not quiet:
            print('Parameters',parameter)
            print(s['species'])
            print(X0)

kappa_r_2 = 1
X0_A = 1
X0_B0 = 1
X0_E = 0.01
X0_C = 0.01
```

5.3 Transfer function analysis

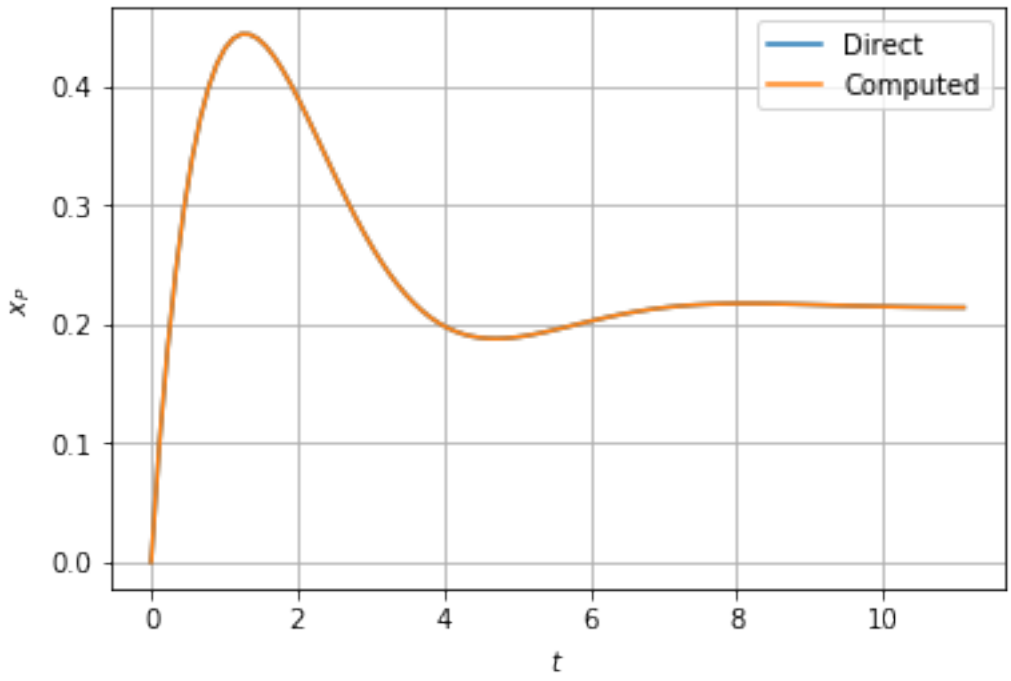
```
In [14]: t_step = np.linspace(0,1000,100)
         t_step = None
         t_ss = 1e2

         x_ss,v_ss,CL = ClosedLoop(Allosteric,chemostats,parameter,X0,t_ss,t_step,quiet=quiet)

         CLL,L = LoopGain(Allosteric,chemostats,parameter,x_ss,quiet=quiet)

         print('Closed-loop: direct')
         showTF(CL)
         print('Closed-loop: from loop-gain')
         showTF(CLL)

         ## Find reduced-order systems using balanced reduction
         print('Closed-loop: direct (reduced)')
         showTF(con.tf(con.balred(con.tf2ss(CL),3)))
         print('Closed-loop: from loop-gain (reduced)')
         showTF(con.tf(con.balred(con.tf2ss(CLL),3)))

         t_CL,y_CL = plotCL(CL,CLL,t_step)

Closed-loop. Order =  6
2 states have been removed from the model
Open-loop. Order =  5
4 states have been removed from the model
```

```
Feedback from P to  rc

13.45 s - 0.006295
------------------
    s + 735.1

        gain: -0.000
        poles: [-735.097]
        zeros: [0.]


2 states have been removed from the model

Feedback from P to  re

0.02689 s^3 + 2.699e+04 s^2 + 2.701e+07 s + 6.469e+06
-----------------------------------------------------
    s^3 + 1.004e+06 s^2 + 1.743e+09 s + 7.386e+11

        gain: 0.000
        poles: [-1.003e+06 -1.002e+03 -7.351e+02]
        zeros: [-1.003e+06 -1.002e+03 -2.396e-01]


3 states have been removed from the model

Active feedback from P to r_1

    3.659
-------------
s^2 + 3 s + 1

        gain: 3.659
        poles: [-2.618 -0.382]
        zeros: []



Passive feedback from P to r_1

s^2 + 1.995 s
-------------
s^2 + 3 s + 1

        gain: 0.000
        poles: [-2.618 -0.382]
        zeros: [-1.995  0.   ]
```

```
Feedback from P to  r_1

s^2 + 1.995 s + 3.659
---------------------
    s^2 + 3 s + 1

        gain: 3.659
        poles: [-2.618 -0.382]
        zeros: [-0.998+1.632j -0.998-1.632j]


5 states have been removed from the model

Feedback from P to  r_2

1
-
1

        gain: 1.000
        poles: []
        zeros: []



Gain from B0 to r_2
5 states have been removed from the model

1
-
1

        gain: 1.000
        poles: []
        zeros: []



Net feedback

15.48 s^3 + 1516 s^2 + 3690 s + 3425
------------------------------------
  s^3 + 738.1 s^2 + 2206 s + 735.1

        gain: 4.659
```

```
        poles: [-7.351e+02 -2.618e+00 -3.820e-01]
        zeros: [-95.462+0.j     -1.237+0.888j  -1.237-0.888j]



Forward gain to P

1
-
s

        gain:   inf
        poles: [0.]
        zeros: []



Closed loop
Closed-loop: direct

     s^3 + 738.1 s^2 + 2206 s + 735.1
----------------------------------------
s^4 + 753.6 s^3 + 3722 s^2 + 4425 s + 3425

        gain: 0.215
        poles: [-7.486e+02+0.j    -3.703e+00+0.j    -6.305e-01+0.915j -6.305e-01-0.915j]
        zeros: [-7.351e+02 -2.618e+00 -3.820e-01]


Closed-loop: from loop-gain

     s^3 + 738.1 s^2 + 2206 s + 735.1
----------------------------------------
s^4 + 753.6 s^3 + 3722 s^2 + 4425 s + 3425

        gain: 0.215
        poles: [-7.486e+02+0.j    -3.703e+00+0.j    -6.305e-01+0.915j -6.305e-01-0.915j]
        zeros: [-7.351e+02 -2.618e+00 -3.820e-01]


Closed-loop: direct (reduced)

  0.9824 s^2 + 2.958 s + 0.9859
---------------------------------
s^3 + 4.978 s^2 + 5.925 s + 4.594

        gain: 0.215
        poles: [-3.717+0.j    -0.631+0.915j -0.631-0.915j]
```

```
        zeros: [-2.629 -0.382]


Closed-loop: from loop-gain (reduced)

  0.9824 s^2 + 2.958 s + 0.9859
---------------------------------
s^3 + 4.978 s^2 + 5.925 s + 4.594

        gain: 0.215
        poles: [-3.717+0.j    -0.631+0.915j -0.631-0.915j]
        zeros: [-2.629 -0.382]
```



5.3.1 Discussion

Transfer function orders are reduced in two ways:

- Using con.minreal (minimal realisation) to cancel approximately identical poles and zeros (using tolerance tol). This is used each time a transfer function is extracted.
- Using con.balred (balanced reduction) to give a transfer function of given order.

In this case there are four transfer functions relating the product concentration x_P to the to the four flows which add to drive the product concentration x_P

- v_{r1} - the main feedback path. This is $\dfrac{s^2 + 1.995s + 3.659}{s^2 + 3s + 1}$
- This is the sum of two transfer functions:
  - an *active* path via the allosteric inhibition module: $\dfrac{3.659}{s^2 + 3s + 1}$
  - a *passive* path via the admittance of the reaction chain at the product **Ce:P**: $\dfrac{s^2 + 1.995s}{s^2 + 3s + 1}$
- v_{r2} - the product flow absorbed by **Ce:B0**. This is the unit transfer function in this case.
- $5v_{re}$ and $5v_{ce}$ - the flow transiently absorbed by **Ce:EM** and **Ce:CM**. With reference to the bode diagram, this is small and can be ignored.

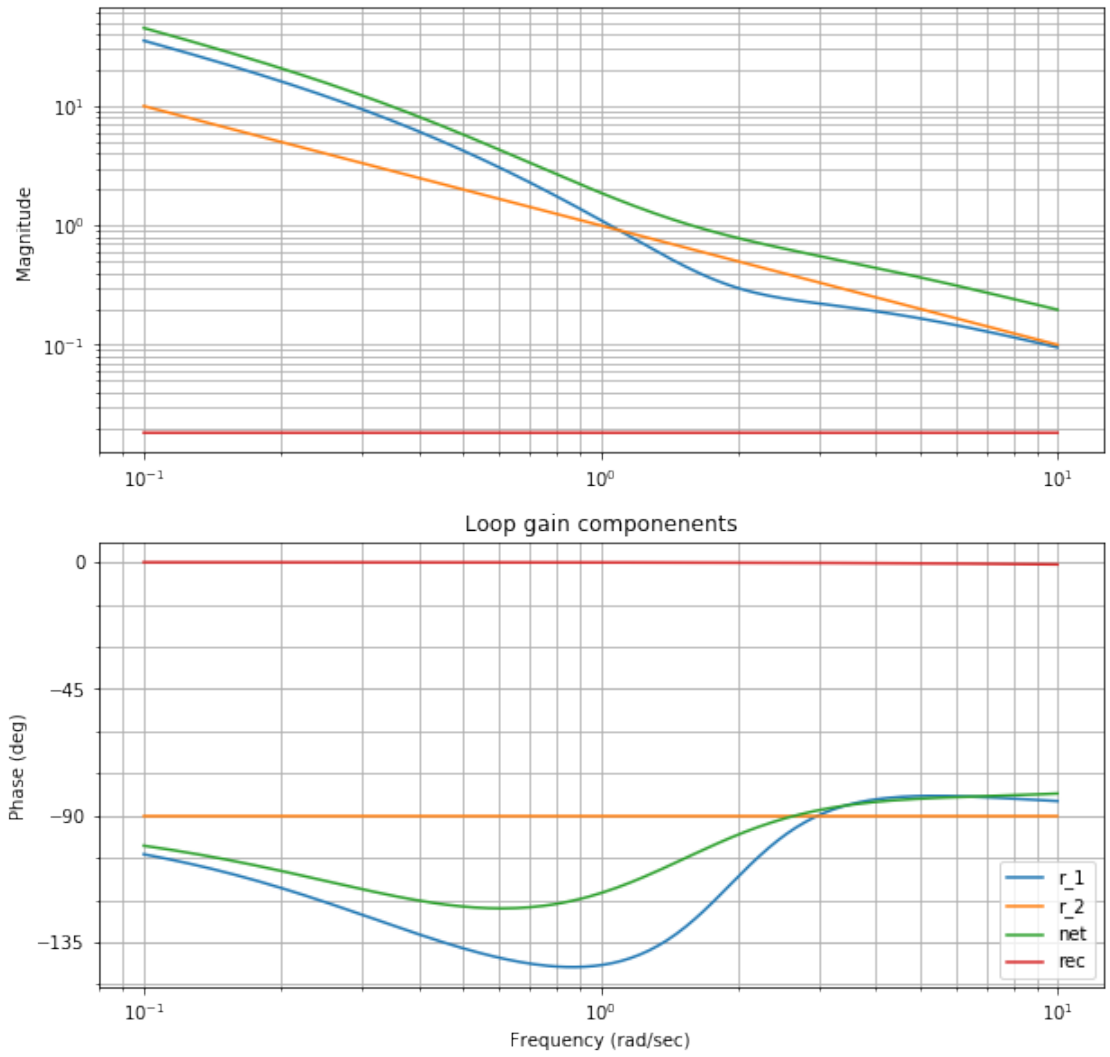
The closed-loop transfer function relating the disturbance concentration x_{B0} to product concentration x_P can be derived in two ways: directly, and from the loop gain. When reduced to third order, the closed-loop transfer function is $\dfrac{0.9824s^2 + 2.958s + 0.9859}{s^3 + 4.978s^2 + 5.925s + 4.594}$.

- This has a real pole at $s = -3.7$ and a complex pair of poles at $s = -0.631 \pm 0.915j$. This gives a stable but oscillatory response.
- the steady-state gain is 0.215. Thus a unit disturbance gives a 21.5% change in product concentration, without feedback this would be 100%.
- The step response plot verifies this.

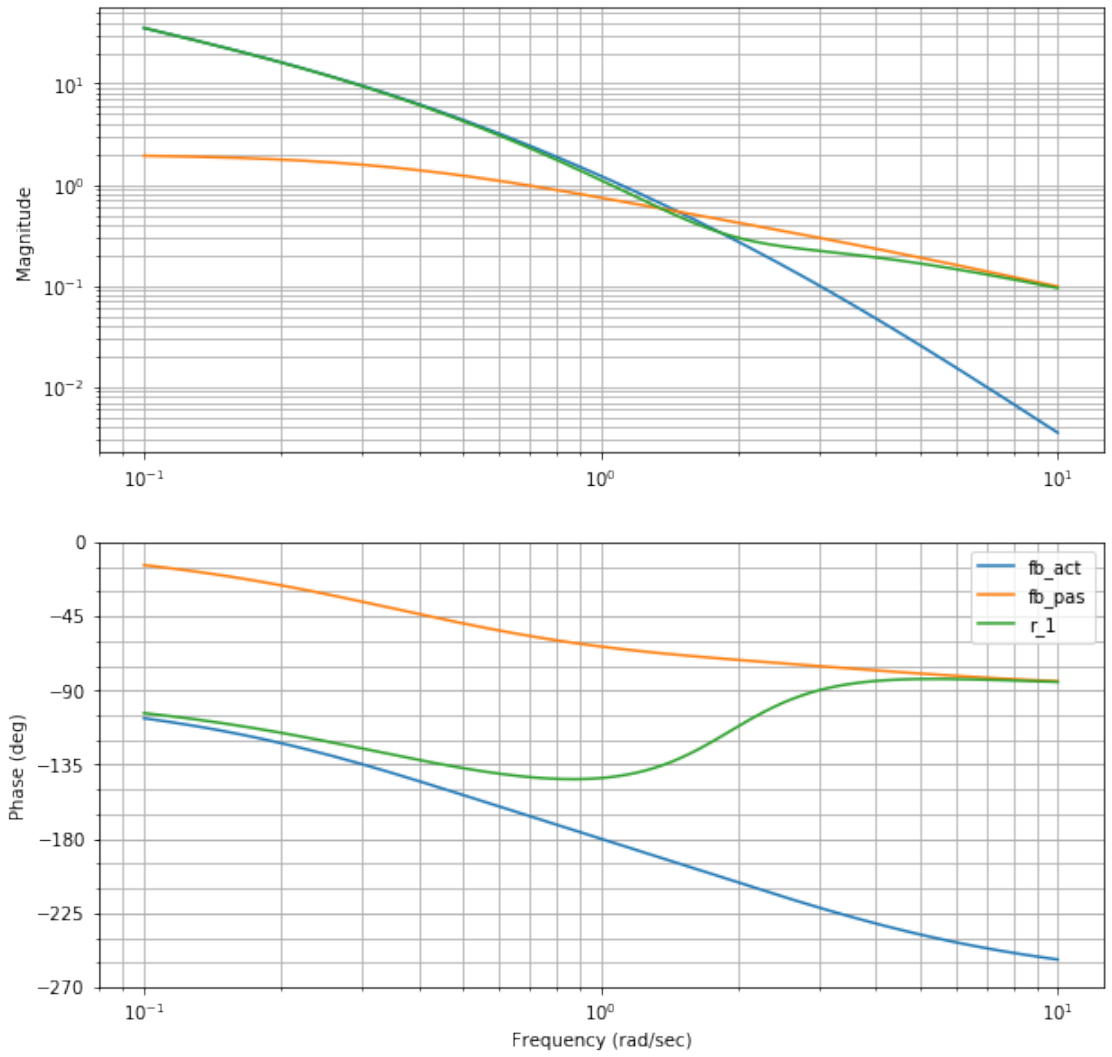
5.4 Frequency response analysis

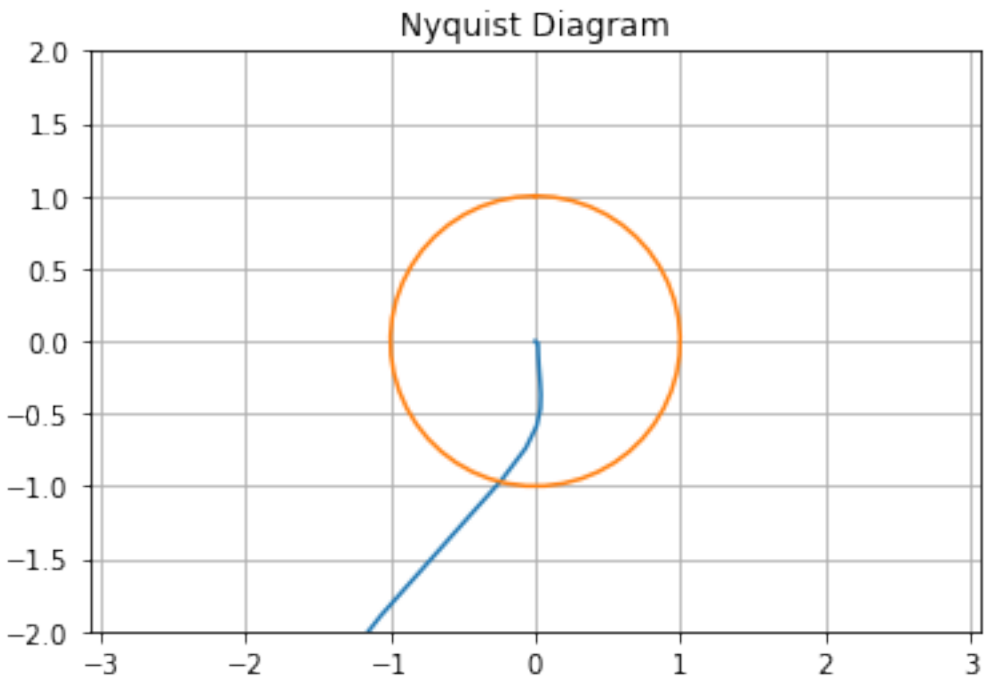
```
In [15]: plotOL(L)
```

```
Bode diagram: loop gain components
```



Bode diagram: Passive and active loop-gain components


Magnitude
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-1}
10^{0}
10^{1}
Phase (deg)
0
−45
−90
−135
−180
−225
−270
fb_act
fb_pas
r_1
Frequency (rad/sec)



5.4.1 Phase margins

```
In [16]: gm, pm, wg, wp = con.margin(L['net'])
         #print('Gain margin',gm)
         print('Phase margin of loop-gain is',pm,'at omega =',wp)

         # gm, pm, wg, wp = con.margin(L['fb_act'])
         # print('Phase margin of fb_act is',pm,'at omega =',wp)

         gm, pm, wg, wp = con.margin(con.parallel(L['fb_act'],L[r_last]))
         print('Phase margin of fb_act+fb_r_last is',pm,'at omega =',wp)
```

```
Phase margin of loop-gain is 75.95006861540071 at omega = 1.581685736613151
Phase margin of fb_act+fb_r_last is 42.172473898898204 at omega = 1.2668858916825174
```

5.4.2 Discussion

- A control systems discussion is needed here
- Active feedback dominates at low fequencies , and passive feedback at high frequencies.
- The active feedback reduces the effect of disturbances at low frequencies
- The passive feedback has no effect at zero frequency but has a phase advance effect at high frequencies thus improving phase margin and thence stability.
- even long reaction chains, which would be destabilised by the active feedback alone, are stabilised by the passive feedback.

5.5 Compare linear and non-linear responses

In [17]:
```
t_max = int(11)
t = np.linspace(0,t_max,1000)
t_0 = 1


AMP = [0.1,1]
Legend = ['Linear']
for amp in AMP:
    ## Chemostat
    x_B0 = x_ss[s['species'].index('B0')]
    x_chemo = '{0}*(1+{2}*np.heaviside(t-{1},1))'.format(str(x_B0),str(t_0),str(amp))
    x_chemo_lin = '{0}*(np.heaviside(t-{1},1))'.format(str(x_B0),str(t_0))
    X_chemo = {'B0':x_chemo}
    X_chemo_lin = {'B0':x_chemo}

    ## Non linear simulation (from steady state)
    ndat = st.sim(s,sc=sc,t=t,parameter=parameter,X0=x_ss,X_chemo=X_chemo,quiet=quiet)
    i_P = s['species'].index('P')
    x_P = ndat['X'][:,i_P] - x_ss[i_P]
    if amp is AMP[0]:
        X_P = x_P/amp
    else:
        X_P = np.vstack((X_P,x_P/amp))
    Legend.append('non-linear, amp = '+str(amp))

##Plot
plt.title('Linear and normalised non-linear step responses')
plt.plot(t_CL,y_CL,linewidth=4,linestyle='--')
plt.plot(t-t_0,X_P.T)
plt.xlabel('$t$')
plt.ylabel('x_P/amp')
plt.legend(Legend)
plt.grid()
plt.show()
```

Out[17]:
```
Text(0.5,1,'Linear and normalised non-linear step responses')
```

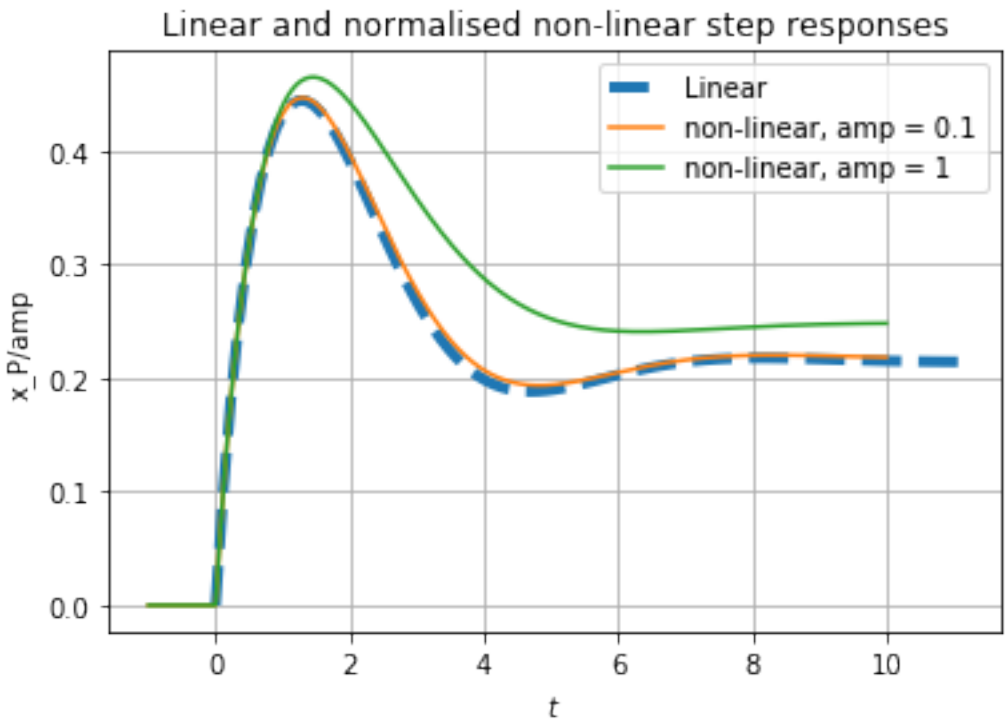
Out[17]:
```
[<matplotlib.lines.Line2D at 0x7fe1342176d8>]
```

Out[17]:
```
[<matplotlib.lines.Line2D at 0x7fe1341647f0>,
 <matplotlib.lines.Line2D at 0x7fe134164978>]
```

Out[17]:
```
Text(0.5,0,'$t$')
```

Out[17]:
```
Text(0,0.5,'x_P/amp')
```

Out[17]:
```
<matplotlib.legend.Legend at 0x7fe149ecf4e0>
```



5.5.1 Discussion

The full non-linear simulation verifies that a disturbance step change of

- 0.1 gives a close fit
- 1.0 gives a reasonable fit.

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