# Chapter 2

## 2.1 Week 1 Activities and Assignments Set 2

This is another set of activities for week 1 following up on"Set 1".

Here we take up examples from GTPase signalling motifs, discussed in [1]. Most of the exercises and activities of this "Set 2" are based on material that is available in short lectures and slides on our website under "GTPases and cell signalling" at the link:

`http://www.math.ubc.ca/~keshet/MCB2012/Week1.html`

Please listen to the short lectures and look at the slides. The diagrams there go with the material below.

## 2.2 Phosphorylation as a way to activate a protein

First, here is some background and definitions of notation. Much of this also appears on the slides.

We refer to $M$ as the concentration of some signalling protein and $M_p$ as its activated phosphorylated form. It will be assumed that the protein is phosphorylated by some kinase, and dephosphorylated by a phosphatase. so that

$$\frac{dM_p}{dt} = v_{kin} - v_{phos} \tag{2.1a}$$

$$\frac{dM}{dt} = -v_{kin} + v_{phos} \tag{2.1b}$$

This means that the total is conserved,

$$M + M_p = M_{tot} = \text{constant}$$

It is generically assumed that in the absence of feedback, each of the above reactions satisfies Michaelis-Menten kinetics. [This is one reason why we reviewed such kinetics, and why it is valuable to be cognizant of what such kinetics represent.]

$$v_{kin} = \frac{V_1 M}{(K_{m1} + M)} \tag{2.2a}$$

$$v_{phos} = \frac{V_2 M_p}{(K_{m2} + M_p)} \tag{2.2b}$$

$$v_{kin} = \frac{(k_{kin}^{cat} E_{kin} M)}{(K_{m1} + M)} \tag{2.3a}$$

$$v_{phos} = \frac{(k_{phos}^{kin} E_{phos} M_p)}{(K_{m2} + M_p)} \tag{2.3b}$$

The above notations have these meanings:

Kinase Parameters:

$k_{kin}^{cat}$= maximal rate of phosphorylation per kinase (1/sec)
$E_{kin}$= Amount of kinase available (nM)
$K_{m1}$= typical concentration of $M$ at which kinase works at half-maximal rate (Michaelian saturation constant) in nM.

Phosphatase parameters:

$k_{phos}^{kin}$= rate of dephosphorylation per kinase (1/sec),
$E_{phos}$= amount of kinase (nM).
$K_{m2}$= typical concentration of $M_p$ at which phosphatase works at half-maximal rate (Michaelian saturation constant) in nM.

We now consider a variety of feedback from the active protein $M_p$ to the other parts of the system. The feedback has implications on the dynamics.

### 2.2.1 A bistable switch

We first consider positive feedback from the active form $M_p$ to the kinase. Consequently, we assume that higher $M_p$ will lead to higher rate $V_{kin}$. This is the first example on the slides

$$v_{kin} = \frac{(k_{kin}^{cat} E_{kin} M)}{(K_{m1} + M)} \frac{(1 + A(M_p/K_a))}{(1 + (M_p/K_a))}$$

The parameter $A > 1$ governs the extent of the feedback effect. The phosphorylation rate is kept as in Eqn. (1.3b).

Here are the kinase Parameter values:
$k_{kin}^{cat} = 1/\text{sec}$, $A = 100$, $K_a = 500\text{nM}$, $K_{m1} = 500$ nM, $E_{kin} = 80\text{nM}$. Phosphatase parameter values: $k_{phos}^{kin} = 1/\text{sec}$, $K_{m2} = 10$ nM, $E_{phos} = 200\text{nM}$.

**Exercise 2.2.1 (Phosphorylation, etc)**

(a) Explain all the terms in the equations, and in particular, how the positive feedback is being modeled.

(b) Simulate this system using your favorite software to show that it behaves like a bistable switch. (Think of ways of doing so based on the experience you gained in HW 1 set 1.) If you'd like to use XPP, there is a short code provided in the Appendix 1.4.1.

(c) The paper by Kholodenko and the slides on our site mention other ways of connecting up the feedback to obtain a bistable switch (i.e. feedback from $M_p$ to some other part of the system.) Pick any ONE other example, show how the model equations are formulated, and modify your code to show that it also behaves like a "switch". Try not to select the same example as your friend -diversity is welcomed!

## 2.3 An oscillating GTPase circuit

Next, we consider a case when the active protein $M_p$ exerts negative feedback on the production of the kinase. This was a second example discussed in the slides.

Now the kinase is also a variable, and thus satisfies its own equation,

$$\frac{dE_{kin}}{dt} = v_{kin}^{synth} - v_{kin}^{deg}$$

The negative feedback from $M_p$ to kinase production is modeled as follows:

$$v_{kin}^{synth} = V_{kin}^{0} \frac{(1 + (M_p/K_l))}{(1 + I(M_p/K_l))}$$

Kinase is degraded at constant rate

$$v_{kin}^{deg} = k_{kin}^{deg} E_{kin}$$

The full model is now as follows:

$$\frac{dM_p}{dt} = v_{kin} - v_{phos} \tag{2.4a}$$

$$\frac{dE_{kin}}{dt} = v_{kin}^{synth} - v_{kin}^{deg} \tag{2.4b}$$

$$v_{kin} = \frac{(k_{kin}^{cat} E_{kin} M)}{(K_{m1} + M)} \frac{(1 + A(M_p/K_a))}{(1 + (M_p/K_a))} \tag{2.4c}$$

$$v_{phos} = \frac{(k_{phos}^{kin} E_{phos} M_p)}{(K_{m2} + M_p)} \tag{2.4d}$$

$$v_{kin}^{synth} = V_{kin}^0 \frac{(1 + (M_p/K_l))}{(1 + I(M_p/K_l))} \tag{2.4e}$$

$$v_{kin}^{deg} = k_{kin}^{deg} E_{kin} \tag{2.4f}$$

with $M + M_p = M_{tot}$=constant, as before.

New parameter values: $V_{kin}^0 = 150$nM/hr, $K_l = 100$nM ,$I = 7.5$, $k_{kin}^{deg} = 1$/hr. Please carefully note the time units.

Then this kind of feedback can set up stable cycles. This is shown in the graphs on the slides.

**Exercise 2.3.2 (Oscillating GPase feedback circuit, etc)**

(a) Explain the new equations, paying particular attention to the way that inhibition is modeled. Note that such systematic modeling building-blocks help to craft increasingly complex models without introducing new arbitrary decisions at each step - that is, positive and negative feedback are always represented in a similar way.

(b) In the slides, we have shown that this circuit can oscillate. An XPP file for this example is given in Appendix 1.4.2. Compare this system with the classic Fitzhugh Nagumo model for excitable kinetics with limit cycle oscillations.

$$\frac{dx}{dt} = c\left(-\frac{x^3}{3} - y + j\right) \tag{2.5a}$$

$$\frac{dy}{dt} = \frac{1}{c}(x + a - by) \tag{2.5b}$$

The shape of the "cubic" nullclines gives rise to the interesting aspects of the system, namely the "excitable" kinetics and the formation of a limit cycle.

An XPP code for simulating this example is provided in Appendix 1.4.3.

(c) Pick an "equivalent" circuit from the examples, modify the model, and show by simulations that it also sustains oscillations.

Comments: The paper by [1] and the follow-up article with a similar flavour in [2] would be suitable as extended project topics in this course.

# Bibliography

[1] B.N. Kholodenko. Cell-signalling dynamics in time and space. *Nature Reviews Molecular Cell Biology*, 7(3):165–176, 2006.

[2] M.A. Tsyganov, W. Kolch, and B.N. Kholodenko. The topology design principles that determine the spatiotemporal dynamics of g-protein cascades. *Mol. BioSyst.*, 8(3):730–743, 2012.

## 2.4 Appendix

### 2.4.1 XPP code for Bistable switch example

```
# Kholod06BistSwitch_a.ode.ode
#
# Kholodenko (2006) Nat Rev Mol Cell Bio 7, p 165
# Box 2 Example (a)
#

Mp'=vkin(Mp,Mtot-Mp)-vphos(Mp,Mtot-Mp)

vkin(Mp,M)=((k_kincat*Ekin*M)/(Km1+M))*((1+A*(Mp/Ka))/(1+(Mp/Ka)))
vphos(Mp,M)=k_phoskin*Ephos*Mp/(Km2+Mp)

par k_kincat=1,A=100,Ka=500,Km1=500,Ekin=80
par k_phoskin=1,Km2=10,Ephos=200
par Mtot=300

@ total=20,xlo=0,xhi=20,ylo=0,yhi=300,bounds=1000
done
```

### 2.4.2 XPP code for oscillating GTPase model

```
# Kholod06Oscill_c.ode
#
```

```
# Kholodenko (2006) Nat Rev Mol Cell Bio 7, p 165
# Box 2 Example (c)
#
# M is protein and Mp is its phosphorylated form
# Ephos is the phosphatase

Mp'=vkin(Mp,Mtot-Mp)-vphos(Mp,Mtot-Mp)
Ephos'=v_phossyn(Mp)-v_phosdeg(Ephos)

vkin(Mp,M)=((k_kincat*Ekin*M)/(Km1+M))*((1+A*(Mp/Ka))/(1+(Mp/Ka)))
vphos(Mp,M)=k_phoscat*Ephos*Mp/(Km2+Mp)

v_phossyn(Mp)=V_phos0*(1+Ap*(Mp/Kd))/(1+(Mp/Kd))
v_phosdeg(Ephos)=k_phosdeg*Ephos

# these params have units that include per sec already
par k_kincat=1,A=100,Ka=500,Km1=500,Ekin=150
par k_phoscat=1,Km2=10
par Mtot=300

#Convert Kholodenko's params to per sec
# par V_phos0=200nm/hr, k_phosdeg=1/hr now in per sec:
par V_phos0=0.0555,Kd=100,Ap=7.5,k_phosdeg=0.000277

init Mp=300,Ephos=100

@ dt=0.01,total=50000,nout=500,xp=Mp,yp=Ephos,xlo=0,xhi=300,ylo=0,yhi=1000,bounds=1000
done
```

### 2.4.3 XPP code for FitzHugh-Nagumo equations

```
#FitzhughNagumo1.ode
# file to produce simulations for FHN model

dx/dt = c*(x-(x^3)/3-y+j)
dy/dt = (x+a-b*y)/c

par j=0,a=0.7,b=0.8,c=3
# Consider either c=3 or c=10
# Parameters should satisfy  1-(2/3)b<a<1,   0<b<1

# Convenient initial conditions:
init x=2.0,y=2.0

@ total=20,xp=x,yp=y,dt=0.01,xlo=-3,xhi=2,ylo=-1,yhi=2
done
```