

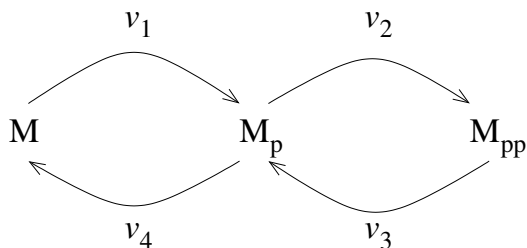
Chemistry 4010 Fall 2019 Assignment 3

Due: Oct. 1, 6:00 p.m.

Total marks: 19 marks

In this assignment, you will use AUTO to obtain a bifurcation diagram and a phase diagram for a model of a MAP kinase (MAPK) [1]. Mitogen-Activated Protein (MAP) kinases are signal-transduction proteins that are activated by phosphorylation by another kinase called a MAP-kinase kinase (MAPKK). Activation of a MAP kinase often requires phosphorylation at more than one site. Typically, the same MAPKK is responsible for all of the phosphorylations of a given MAPK. Phosphorylated MAPK is deactivated by a MAP-kinase phosphatase (MKP).

The following is a diagram of a MAP kinase (M) that is activated by two sequential phosphorylation events:



Each arrow represents an enzyme-catalyzed reaction, with corresponding rates denoted above or below the arrows. M_p is MAPK that has been phosphorylated once, while M_{pp} represents MAPK twice phosphorylated. The two phosphorylation reactions ($M \longrightarrow M_p \longrightarrow M_{pp}$ with rates v_1 and v_2) are catalyzed by the same MAPKK. Similarly, the two dephosphorylation reactions ($M_{pp} \longrightarrow M_p \longrightarrow M$, rates v_3 and v_4) are catalyzed by the same MKP.

1. Write down the rates of change of $[M]$, $[M_p]$ and $[M_{pp}]$ in terms of v_1 , v_2 and v_3 . Use these equations to show that

$$[M] + [M_p] + [M_{pp}] = M_{\text{tot}},$$

where M_{tot} is a constant. Use the latter relationship to eliminate $[M_p]$, leaving you with two differential equations. [3 marks]

2. The rates are given by the following expressions:

$$v_1 = \frac{k_{\text{cat}}^{(1)}[\text{MAPKK}]_{\text{tot}}[\text{M}]/K_{m1}}{1 + [\text{M}]/K_{m1} + [\text{M}_p]/K_{m2}}$$

$$v_2 = \frac{k_{\text{cat}}^{(2)}[\text{MAPKK}]_{\text{tot}}[\text{M}_p]/K_{m2}}{1 + [\text{M}]/K_{m1} + [\text{M}_p]/K_{m2}}$$

$$v_3 = \frac{k_{\text{cat}}^{(3)}[\text{MKP}]_{\text{tot}}[\text{M}_{\text{pp}}]/K_{m3}}{1 + [\text{M}_{\text{pp}}]/K_{m3} + [\text{M}_p]/K_{m4} + [\text{M}]/K_{m5}}$$

$$v_4 = \frac{k_{\text{cat}}^{(4)}[\text{MKP}]_{\text{tot}}[\text{M}_p]/K_{m4}}{1 + [\text{M}_{\text{pp}}]/K_{m3} + [\text{M}_p]/K_{m4} + [\text{M}]/K_{m5}}$$

Suppose that, initially, all of the MAP kinase is in the unphosphorylated form, M, i.e. $[\text{M}](0) = \text{M}_{\text{tot}}$. Moreover, suppose that the MAP-kinase kinase (MAPKK) is unavailable, i.e. $[\text{MAPKK}]_{\text{tot}} = 0$. (The MAP-kinase kinase (MAPKK) itself often needs to be activated by... a MAPKK kinase, or MAPKKK. If the MAPKK hasn't been activated by its MAPKKK, then the amount of active MAPKK would be zero.) What are the coordinates of the equilibrium point in this case? [1 mark]

3. A set of suggested¹ parameters is given in Table 1. The previous question suggests an appropriate initial point for a bifurcation analysis, namely $[\text{MAPKK}]_{\text{tot}} = 0$ with the initial concentrations set accordingly. Using AUTO, obtain a bifurcation curve for this model plotting $[\text{M}_{\text{pp}}]$ vs the bifurcation parameter $[\text{MAPKK}]_{\text{tot}}$. [10 marks]

You should include your XPPAUT input file as well as your graph in your report.

Hints:

- In your XPPAUT input file, you can save yourself some typing by writing your ODEs in terms of v_1 , v_2 , v_3 and v_4 , and then defining these quantities. For example, you can have the following in your ODE file:

$$v1=kcat1*MAPKKtot*M/Km1/(1+M/Km1+Mp/Km2)$$

¹If, for some reason, you decide to use different parameters, make sure you make that clear in your assignment.

Table 1: Suggested parameters for the MAPK model

| Parameter | Value | Units | | | |
|------------------|-------|-------|-----------------------------|------|-----------------|
| K_{m1} | 50 | nM | $k_{\text{cat}}^{(1)}$ | 0.01 | s^{-1} |
| K_{m2} | 500 | nM | $k_{\text{cat}}^{(2)}$ | 15 | s^{-1} |
| K_{m3} | 20 | nM | $k_{\text{cat}}^{(3)}$ | 0.08 | s^{-1} |
| K_{m4} | 20 | nM | $k_{\text{cat}}^{(4)}$ | 0.06 | s^{-1} |
| K_{m5} | 60 | nM | | | |
| M_{tot} | 500 | nM | $[\text{MKP}]_{\text{tot}}$ | 100 | nM |

The definition of an intermediate quantity like $\mathbf{v1}$ that is used to compute the right-hand side of an ODE is called a “hidden quantity” in XPPAUT. Note that the expression for $\mathbf{v1}$ includes \mathbf{Mp} , which we were planning to eliminate. You can use another hidden quantity to define \mathbf{Mp} **provided it appears before $\mathbf{v1}$** , which contains this variable. In general, a hidden quantity must be defined before it appears in another hidden quantity.

- If you want to integrate the differential equations, you need to adjust the `BOUNDS`. One easy way to do this is to include a line such as the following in your XPPAUT input file:
`@ BOUNDS=1000`
- You will also find that the differential equations are stiff, so you need to specify an integrator that is able to handle stiff equations:
`@ METH=stiff`
- When you start up AUTO, make sure that both `MAPKKtot` and `MKPtot` are listed among the parameters available since we will later generate a phase diagram using these two parameters.
- The final bifurcation diagram should show two saddle-node bifurcations.
- Once you have a bifurcation diagram, it would be a good idea to save it. This will enable you to load it back in and get back to where you were if something goes wrong. To save your bifurcation diagram, click on `File`→`Save diagram` in the AUTO window.

- You can either do a screen grab to extract your bifurcation diagram, or you can use **File**→**Postscript** to export a Postscript version of your diagram. Another possibility is to export an SVG file from AUTO. SVG files can be viewed in web browsers.
4. Now that you have a bifurcation diagram, generate a phase diagram showing the bistable region in the $([\text{MAPKK}]_{\text{tot}}, [\text{MKP}]_{\text{tot}})$ parameter plane. Make sure to label (by hand, or by editing your figure in a graphics program) in which region of your diagram bistability will be found. [5 marks]

Hints:

- Before you start, make a note of which points in your bifurcation diagram are the saddle-node points.
- In this case, you will need to grab each saddle-node point in turn, and run the two-parameter calculation in both directions from each point to generate the boundaries of the bistable region.
- You can save your phase diagram after computing it. I would suggest you use a different file name than for the bifurcation diagram so you can recall either later, as needed.

References

- [1] Nick I. Markevich, Jan B. Hoek, and Boris N. Kholodenko. Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades. *J. Cell Biol.*, 164:353–359, 2004.