Problem Set #2
MB&B 361/562
Spring 2023
DUE: Friday, February 16, 2024, by midnight
(Paper presenters have until Monday 2/19 at midnight)

Please include your written answers into the LiveScript so you just hand in one pdf.
You may consult with your classmates and the TAs, but all scripts and answers must be your own. We will look at the scripts and answers closely 🕵.

[20 pts for 361 (undergraduates), 25 pts for 562 (grad students)]

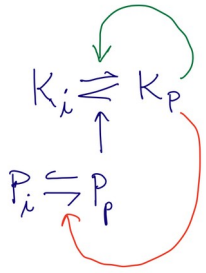
"Circadian rhythms allow organisms to coordinate their lives according to the alteration of their environments by day and by night" (Nakajima, M. *et al.* Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation in vitro. *Science* **308**, 414–415 (2005)). For example, cyanobacteria, one of the simplest organisms that exhibits circadian rhythms, is photosynthetic and only needs its photosynthetic pathway during the days. Using its circadian rhythm, it can anticipate daybreak and be ready to go. In most eukaryotic model organisms, circadian oscillations are due to negative feedback regulation of the expression of "clock" genes. Cyanobacteria are an exception: Kondo et al. (paper attached) showed that the cyclic phosphorylation of KaiC, one of the key regulatory proteins, can be reconstituted in the test tube with just three proteins KaiC, KaiA and KaiB (plus ATP). Thus, gene regulation is not essential, though subsequent work shows that it does play a role in the clock.

Read the Nakajima *et al.* paper (available with this homework) and answer the following two questions:
(a) [1 pt] From Figures 1c and 2a, estimate the oscillation period.
(b) [1 pt] Mutant bacteria that have cycling periods significantly differently from 24 hours have a competitive disadvantage compared to those with periods close to 24 hours. Why do you think this is?

We are going to build a very simple model of the oscillation. This general mechanism of oscillation crops up in many places; it is an example of a relaxation oscillator.

Based on experiments in living cells, Kondo and colleagues have hypothesized that the oscillation comes about through autophosphorylation and auto-dephosphorylation activities of KaiC. These activities are modified by KaiA and KaiB, but we are going to ignore this for the moment.

The model that we are going to develop has KaiC with a kinase domain (K) and a phosphatase domain P. The phosphorylated kinase domain K_P phosphorylates itself and also phosphorylates the phosphatase domain to activate it:



The idea behind the model is that we convert a bistable system, the top half of the diagram above (similar to Homework 1), by adding a slow feedback on the dephosphorylation that brings the system back towards its unstable point.

For the autophosphorylation, we assume the model specified by:

$$\dot{K}_P = \left(b \frac{{K_P}^n}{{K_M}^n + {K_P}^n} + 0.1 \right) \left(K_{tot} - K_P \right) - P_P \cdot K_P \qquad \text{(Equation 1)}$$

where K_P is the active (phosphorylated) kinase domain of KaiC. This corresponds to the green pathway (above). P_P is the active (phosphorylated) phosphatase domain of KaiC.
Some useful lines of code:

```
Ktot = 3                  %Total kinase (phosph. and unphosph.)
K_P = [0:Ktot/100:Ktot]; %Phosphorylated (active) kinase
K_M=1;                    %K_M for activating of the kinase & phosphatase
b=0.8;                    %scaling in dK_P/dt
n=6;                      %cooperativity

% the dKP nullcline (Psolve):
Psolve = (b.*K_P.^n./(K_M^n + K_P.^n)+0.1).*(Ktot -K_P)./K_P;
```

(c) [2 pts] Plot the nullcline corresponding to $\dot{K}_P = 0$, with K_P on the *y*-axis and P_P on the *x*-axis (remember from the Problem Set 1 that that it is easy to write $P_P(K_P)$).

For the autodephosphorylation, we want to make the autophosphatase activity of KaiC's phosphatase domain, P, a dynamic variable that depends on K_P (i.e. the phosphorylated, active KaiC kinase domain). This corresponds to the red pathway above:

$$\dot{P}_P = k \left(a \frac{{K_P}^6}{{K_M}^6 + {K_P}^6} - P_P \right) \qquad \text{Equation 2}$$

Code:

```
a=2;                      %scaling in dP_P/dt
```

For simplicity, we will assume that only a small percentage of the phosphatase domain is phosphorylated ($P_P \ll P_{tot}$). We want k to be very small so the timescale of the change in P_P is much slower than the equilibration timescale for K_P (but this will be done below): k sets the period of the oscillation.

(d) [2 pts] Plot the nullcline corresponding to $\dot{P}_P = 0$ on the same Figure as (i).

Helpful code:

```
% now plot the dP_P nullcline:
plot(a*K_P.^n./(K_M^n+K_P.^n),K_P,'r-','linewidth',2);
```

(e) [1 pt] Find the fixed point.
(f) [1 pt] Is the fixed point stable or unstable?
(g) [1 pt] What are the eigenvalues of the Jacobian? Remembering that k is small, is the slow or fast time scale unstable (expand the square root under the assumption $k \ll 1$)?

(h) [4 pts] Verify numerically (with ODE45) with these values of K_M and a, that Equations 1 and 2 oscillate. k needs to be small to have a slow oscillation. As a trial value let $k = 0.001$. Find a value of k (per minute) that gives a period of oscillation of 24 hours = 1440 minutes. Hand in a plot of P and K_P as functions of time over several periods with this value of k.
Helpful code:

```
dydt = @(t,y)[(Ktot-y(1)).*(b.*y(1).^n./(K_M^n + y(1).^n)+0.1)-y(2).*y(1);
...
    k*(a.*y(1).^n./(K_M^n + y(1).^n)-y(2))];
[T,Y] = ode45(dydt,[0,10000],[1.25 0.75]);
```

(i) [2 pts] Now plot a phase-plane plot of $K_P(t)$ (y-axis) plotted against $P_P(t)$ (x-axis). On the phase plane plot, also plot the nullclines from parts (i) and (ii). Use a legend to identify all 3 curves (trajectory + 2 null clines).
(j) [1 pt] Draw 4 arrows to indicate the direction of motion.
(k) [1 pt] Which nullcline is the solution hugging?
(l) [1 pt] Why that nullcline and not the other?
(m) [1pt] *[bonus for 361; required for 562 (graduate students)]* Plot the vector field using `quiver` (from the Collins MATLAB script in the Tuesday (1/31) script). Because the P_P-component is so small, you will have to rescale the P_P-component of the vector 100 to 1000 times to see what direction the arrow is pointing.

(n) [2 pts] Any idea what KaiA and KaiB could be doing? There is no correct answer – I am looking for some good ideas.

(o) [4 pts] *[bonus for 361; required for 562 (graduate students)]* Prove that a limit cycle exists for the parameters chosen. *Hint*: calculate the vector in the P_P-K_P plane and use the Poincare-Bendixson Theorem.