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Modeling the Mechanism of Circadian KaiC Phosphorylation in Cyanobacterium Synechococcus elongatus

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By

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This Senior Honors Project:

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has been approved.

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Project Advisor date

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Abstract

Until recently, it was believed that the mechanisms for circadian rhythm in all organisms were very complex, requiring many cofactors which were difficult to identify. However, Nakajima *et al.* showed that one organism, the cyanobacterium *Synechococcus elongatus,* contains three clock proteins, KaiA, KaiB, and KaiC, that, when purified and mixed with ATP, produce circadian oscillations *in vitro*[1]. In this project, an attempt to determine the mechanism for these circadian oscillations is made; building on previously published work[2] of Dr. David Lubensky, professor at the University of Michigan. The approach relies on relatively simple mass-action kinetics to model the system. Two adaptions of the van Zon model[2] were attempted: slowing the binding rate of KaiB and introducing a dimer/tetramer interaction to the model. Unfortunately, both of these mechanisms failed to model the experimental oscillating system. Proving that this simple model is most likely not the mechanism controlling this phenomenon.

Background

Circadian rhythm is a specific type of chronobiological function which can be seen in a diverse range of organisms. In order to classify as "circadian", a biological function must fulfill four criteria: 1) it must have approximately a 24 hour period 2) it must be entrainable, meaning it can adjust to external cues, 3) it must be endogenous, meaning it takes place in the absence of external cues, 4) it must exhibit heat compensation, meaning that the function resists dramatically changing rate with dramatic changes of temperature[3]. Cyanobacteria, also known as blue-green algae, are the simplest and most recently discovered form of life known to exhibit circadian rhythms. Until the mid-1980s, it was believed to be impossible for any prokaryotic organism to exhibit circadian behavior. This is because most active prokaryotic cells divide many times in a 24 hour period, making a 24 hour control mechanism seem unnecessary if not impossible. However, in 1986 it was discovered that the prokaryotic organism *Synechococcus sp.* has a nitrogen fixing cycle which qualified as circadian and continues through generations of cells[4,5]. The discovery of that organism led to the research and discovery of several circadian prokaryotes including the organism of interest in this work, *S. elongatus*.

Since the discovery of the *in vitro* Kai system there have been numerous theories and models published to describe the mechanism. As there is not yet a way to observe the individual reactions and interactions of the Kai proteins, one must turn to numerical methods to attempt to discover the mechanism. The *in vitro* Kai system is ideal for modelers as it appears to be rather simple, containing only four components (the 3 proteins and ATP). Also, a special feature of this system is the wealth of experimental data which places constraints on the model. Markson and O'Shea give a review[6] of several proposed models for the system, all of which are based on experimental data. Nakajima *et al.*[1] showed that the oscillations in time are fluctuations in the fraction of total KaiC phosphorylated. It is believed that this phosphorylation is the engine controlling the global cell rhythmic processes. Other groups have discovered that KaiC has both autophosphatase and weaker autokinase properties[7-12]. KaiA acts as a

stimulant to phosphorylation of KaiC[8,9,13] while the presence of KaiB counteracts this effect[8-10]. Experiments have also been able to characterize the individual proteins' shapes. It has been shown that the three proteins have very different quaternary structures: KaiA is a dimer, KaiC is a hexamer[13], and KaiB can exist as either a dimer or tetramer[13-15]. In this project, the previously existing model created by von Zon *et al.* was revisited in an effort to better match experimental data.

All data and plots in this report were created computationally using an adaption of the von Zon model[2] in MATLAB. Unless otherwise noted, all figures were created by the author and are original.

Current Model

The current model[2] theorizes that each of the six monomers in the KaiC hexamer are capable of being phosphorylated. The mechanism for oscillation between phosphorylated and unphosphorylated KaiC is based on a hypothesis that KaiC can exist in two separate conformational states, an active and an inactive state by convention. The active state is more likely to become phosphorylated and the inactive state favors dephosphorylation (fig. 1). To facilitate the circulation, as KaiC becomes more phosphorylated, it becomes more thermodynamically favorable for it to "flip" to the inactive state, designated with a hat, with rate f^n. Likewise as KaiC loses phosphate groups it becomes favorable to "backflip" to the active state with rate b^n. The superscript refers to the number of phosphate ions bonded to the KaiC hexamer. These states facilitate the (de)phosphorylation by altering the way in which KaiC interacts with KaiA and KaiB. In the active state, KaiA acts as a catalyst, stimulating the phosphorylation of KaiC

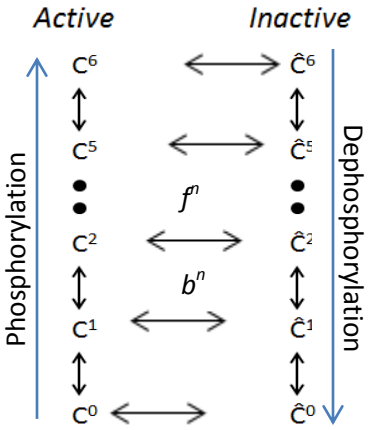

Figure 1: Simplified diagram of circulation of KaiC showing active and inactive states adapted from van Zon[2]. Here the superscript refers to the phosphorylation state of KaiC.

$$C^n + A \xrightarrow{K^n_{ps}} C^{n+1} + A. \qquad (1)$$

where K^n_{ps} is the rate constant for the phosphorylation of KaiC, the ratio of the rate of the forward reaction to the reverse reaction. In this equation, and for the remainder of the paper, *C* refers to a single KaiC monomer while *A* and *B* refer to two KaiA and KaiB proteins respectively. It has been shown that for each KaiC hexamer, two KaiA and KaiB proteins interact to form a complex. Also, capital rate constants refer to the ratio of forward and reverse reactions for the remained of the paper. This catalytic action of A is caused by the quick binding and unbinding of KaiA to KaiC, such that eq. 1 is the summation of equations 2 through 4.

$$C^n + A \xrightarrow{K^n_{Af}} AC^n \qquad (2)$$

$$AC^n \xrightarrow{K_{ps}^n} AC^{n+1} \quad (3)$$

$$AC^{n+1} \xrightarrow{K_{Ab}^n} C^{n+1} + A \quad (4)$$

The rate K^n_{ps} is so much slower than K^n_{Af} and K^n_{Ab} (table 1) that the on and off rate of *A* can essentially be ignored. Once the KaiC becomes sufficiently phosphorylated to flip to the inactive state, it is capable of forming a complex with 2 KaiB dimers, allowing KaiC's natural autophosphatase properties to dominate, causing dephosphorylation:

$$\hat{C}^n + B \xrightarrow{\hat{K}_B^n} B\hat{C}^n \quad (5)$$

$$B\hat{C}^n \xrightarrow{\hat{K}_{dps}^n} B\hat{C}^{n-1} \quad (6)$$

In order to oscillate there must be a mechanism which slows down the system of reactions in order to stop it from reaching an equilibrium phosphorylation state. The current method proposes that KaiA binding is very fast, causing phosphorylation to occur quickly once in the active state. The slow-down is caused by the KaiBC complex sequestering 2 KaiA by forming a KaiABC complex:

$$B\hat{C}^n + A \xrightarrow{\hat{K}_A^n} AB\hat{C}^n \quad (7)$$

This essentially lowers the usable concentration of KaiA, while still allowing the same rate of dephosphorylation of KaiC

$$AB\hat{C}^n \xrightarrow{\hat{K}_{dps}^n} AB\hat{C}^{n-1} \quad (8)$$

The current model also projects a "differential affinity", which assumes that KaiA is only capable of bonding to KaiBC^1-BC^4 (fig.2). The parameter values of this configuration are given in table 1. This

combination of standards allowed for the cyclic pattern of (de)phosphorylation required to produce circadian rhythms (fig. 3).

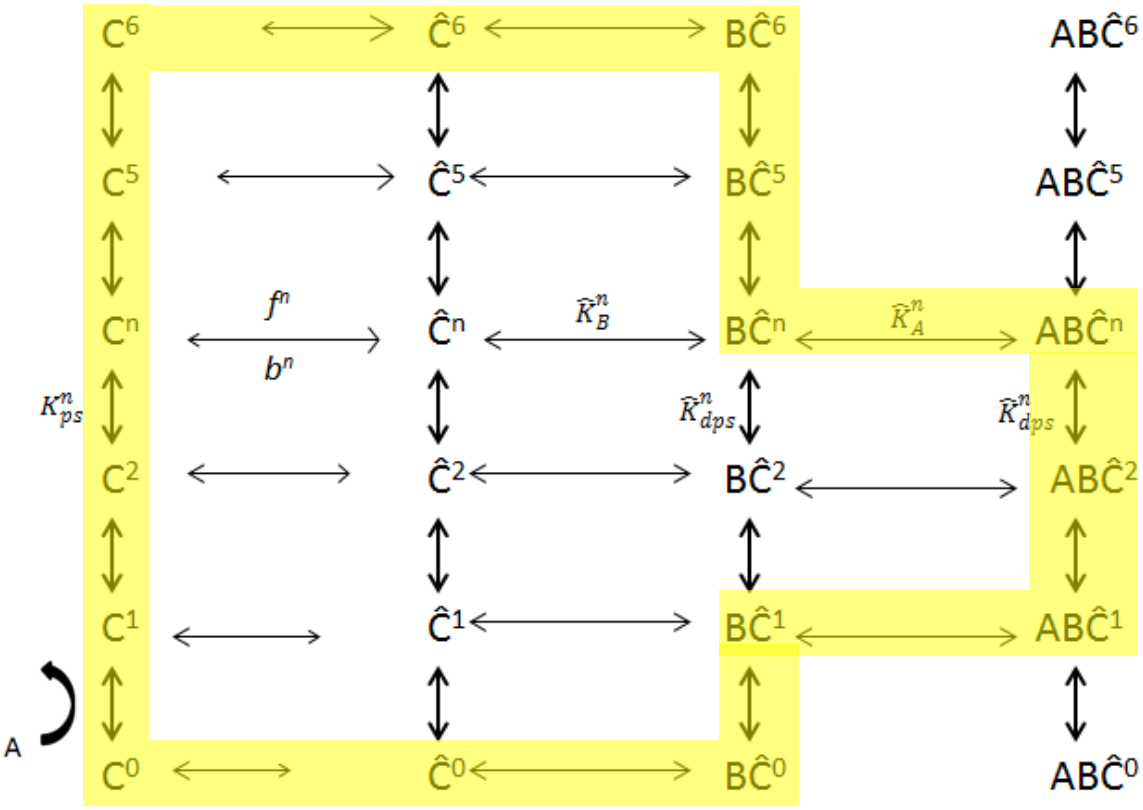

Figure 2: Visual representation of current model with parameter values marked. KaiA acts as a catalyst in the phosphorylation of KaiC. The thermodynamic favorability in flipping/back-flipping is visually represented in changes in the sizes and location of the reaction arrows. It is important to notice that the dephosphorylation rate of KaiC is the same, regardless of the complexation of KaiC. The current model is built so that the majority of KaiC follows the general highlighted path of being phosphorylated completely from base form C^0 to C^6 then fliping to the inactive state, $\hat{C}^6$, and immediately forms a complex, $B\hat{C}^6$. It then loses to phosphate groups before sequestering two KaiA proteins to form $AB\hat{C}^4$. This complex then loses all but one phosphate before breaking the bond with KaiA and losing its final phosphate group. The ground state the loses the associated KaiB proteins and "backflips" to the active state to restart the whole process.

Table 1: Parameter values in current model.[2] Some values are given as vectors because each phosphorylation state of KaiC has its own rate constant. Those values that are given as one term are independent of phosphorylation state.

K_{ps}^n	Phosphorylation Rate	0.025 hr^{-1}
f^n	Flip rate	[10^{-5} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10] hr^{-1}
b^n	Back-flip rate	100 hr^{-1}
k_{Af}^n	Catalytic KaiA Binding rate	1.72x10^{18} $M^{-1}hr^{-1}$
k_{Ab}^n	Catalytic KaiA unbinding rate	[30 60 90 270 810 2430 7290] hr^{-1}
$\widehat{K}_B^n$	KaiB Binding rate	2.97x10^{12} x [0.1 100 100 100 100 100 100] M^{-2}
$\widehat{K}_A^n$	KaiA Binding rate	2.97x10^{16} x [0 1 100 100 1 0 0] M^{-2}
$\widehat{K}_{dps}^n$	Dephosphorylation rate	0.4 hr^{-1}

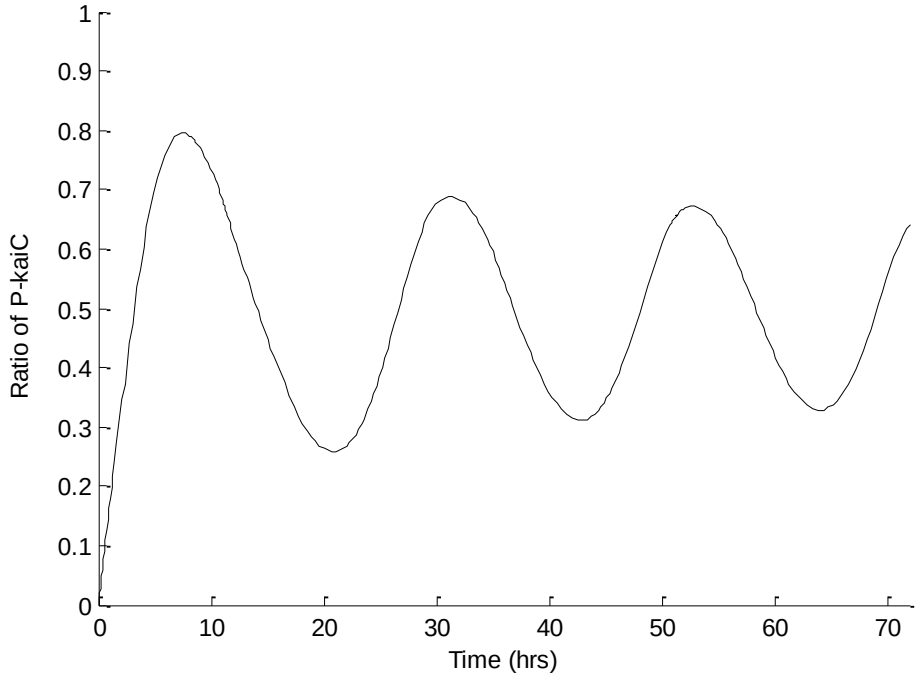

Figure 3: Oscillations in the ratio of phosphorylated KaiC from model described in figure 2 with parameters from table 1.

Lowering KaiB Binding Rate

The von Zon *et al.* model was published in 2006 and was, at the time, taken as plausible explanation. However, as further experiments have been conducted, it has been shown that the rate at which KaiB and KaiC interact to form a complex in the current model, $\widehat{K}_B^n$, is much too fast. There also has never been evidence to suggest that the differential affinity between KaiA and KaiBC actually exists. The first question asked in this project is whether or not simply lowering the rate of complexation of KaiBC is sufficient for causing the circadian rhythm in KaiC phosphorylation without adding differential affinity. Thus, the original model was altered to introduce the reactions of KaiBC^{0,5,6} with KaiA. Once these interactions were added, the binding rate of KaiB was explored. In order to save time, several small, looping programs were written to sweep through a large number of values for KaiB binding at a time. In the end it was realized that for this particular set of parameters there was no value of $\widehat{K}_B^n$ that could cause the model to oscillate, instead, the best results resembled damped oscillators (fig. 4) while most values returned no oscillations at all, or oscillations with periods much longer than 3 days (fig. 5). Thus other parameters were altered in an attempt to make the oscillations appear.

Results and Discussion

In the end, oscillations were attained that were nearly identical to those from the original model as seen in figure 6. In order to attain this result, it was discovered that it was the flip rates which played the biggest role in the oscillations. Once the flip rates were altered, it was discovered that $\widehat{K}_B^n$could remain almost the same. However, the rates of the reaction were slowed drastically. As $\widehat{K}_B^n$ is the ratio between the rates of the forward and reverse reactions, the rate was able to be slowed to a constant forward rate of 0.1uM^{-2}hr^{-l} as opposed to the original rate of 2.97x10^{12}M^{-2}hr^{-1}. The reverse rate was merely adjusted to keep the original rates the same for all values of $\widehat{K}_B^n$ except $\widehat{K}_B^0$ in which the unbinding rate was increased dramatically in order to effectively “kick” the 2 KaiB proteins off of KaiC when it became completely dephosphorylated. There is no known, logical, physical reason for this to

occur, however, this is unimportant as even with the model being shown to work with a parameter set with slow KaiB binding, the model is still incapable of reproducing experimental data. Most importantly, the model is heavily dependent on KaiB concentration. Nakajima, *et. al.* published a paper in 2010 showing there becomes a saturation point, around 3 times the concentration of KaiA, in which there is little to no effect of adding more KaiB. Figure 7 shows the model solution with various concentrations of KaiB, clearly showing a significant difference. After KaiB reaches its peak period at around 3[KaiA], an increase causes a change of over 20% before the model "breaks", meaning it no longer holds steady oscillations, around 5.5[KaiA]. This means that even though it is possible to find a parameter set that works with slow KaiB binding, it is unlikely that this is the main mechanism which is responsible for the oscillations.

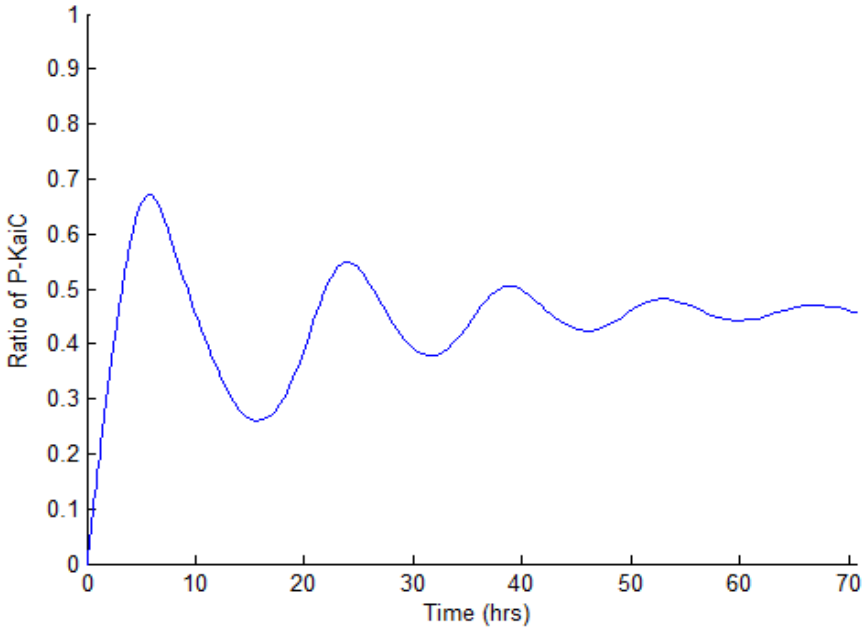

Figure 4: Oscillations resembling a damped oscillator. Altering KaiB binding rate caused KaiC to reach an equilibrium point of phosphorylated KaiC

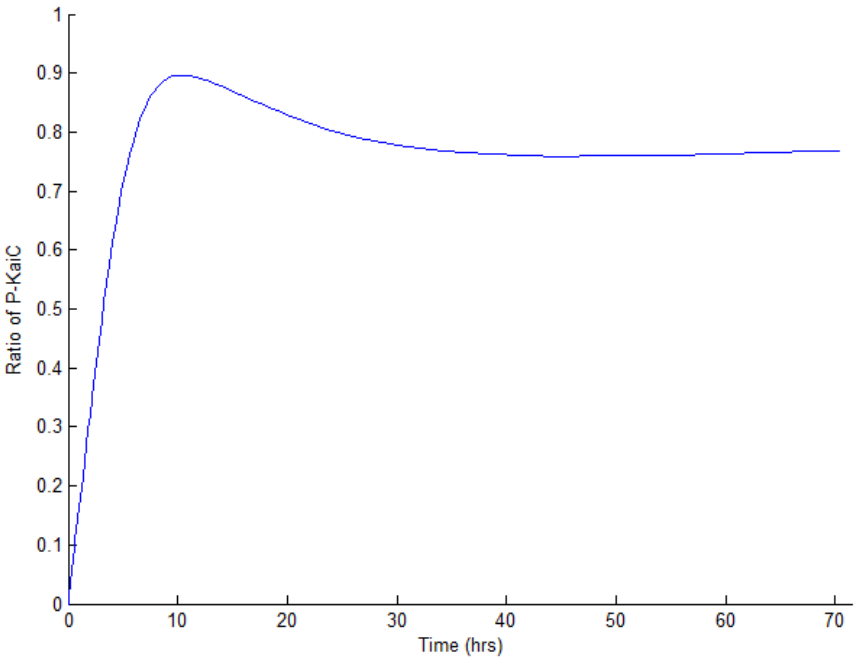

Figure 5: Model results after eliminating the differential affinity from the original model. The period of the oscillation is not possible given the results of experimental data in the literature.

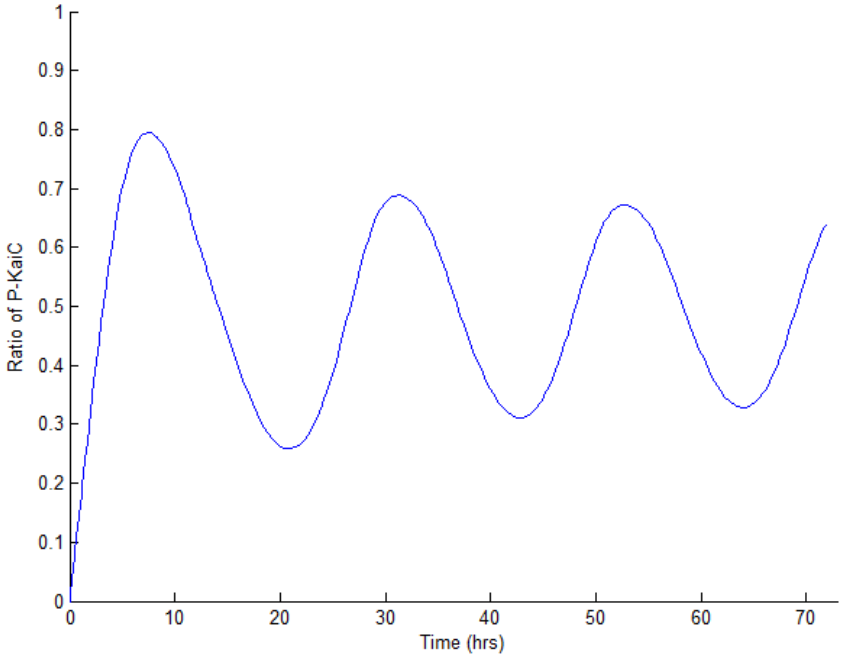

Figure 6: Working model with slow KaiB binding rate.

Table 2: Parameters for working model with slow KaiB binding as seen in figure 6

K_{ps}^n	Phosphorylation Rate	0.025 hr^{-1}
f^n	Flip rate	[1x10^{-8} 1x10^{-6} 1x10^{--4} 1x10^{-3} 0.1 1 10] hr^{-1}
b^n	Back-flip rate	[100 100 10 0.1 1x10^{-2} 1x10^{-4} 1x10^{-6}] hr^{-1}
K_{Af}^n	Catalytic KaiA Binding rate	1.72x10^{18} $M^{-1}hr^{-1}$
K_{Ab}^n	Catalytic KaiA unbinding rate	[30 60 90 270 810 2430 7290] hr^{-1}
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$\widehat{K}_{dps}^n$	Dephosphorylation rate	0.4 hr^{-1}

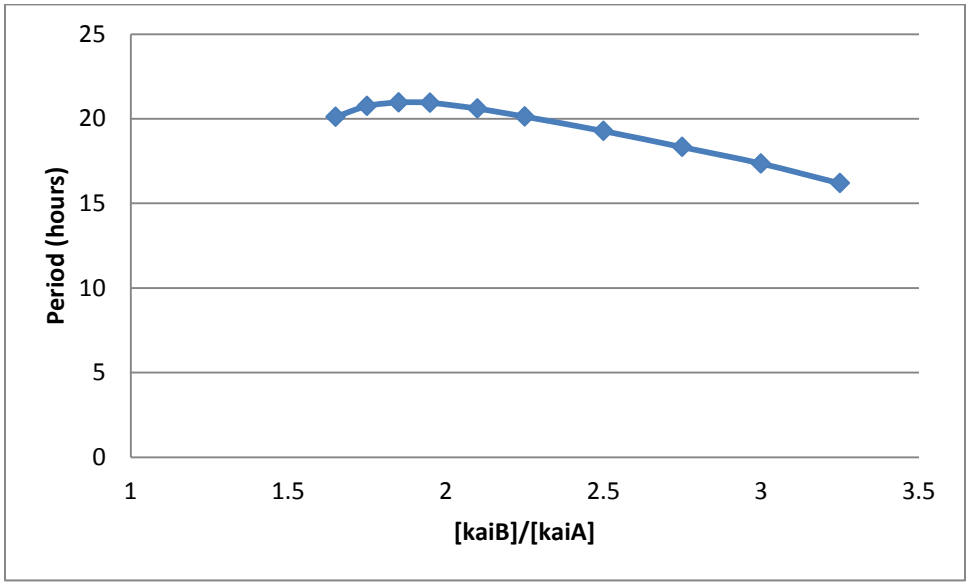

Figure 7: Period dependence on normalized concentration of KaiB from working model. There should be a negligible change once [KaiB] >= 3[KaiA]. Data taken in MATLAB and plotted in Microsoft Excel

Dimer/Tetramer Structural Effects

After slowing the rate of KaiB binding was insufficient in reproducing the experimental data in the system of interest, an alteration to the mechanism was hypothesized. Recently there has been much debate about the active structure of KaiB. There has recently been much debate and disagreement between sources regarding the predominant and active forms of the KaiB protein[15] (also personal communications with Dr. Andrew LiWang, University of California, Merced, July, 2012). One thought is that KaiB favors being in a tetrameric state, but can only interact with KaiC as a dimer. LiWang believes that, at any given point, around 80% of free KaiB is in the form of a tetramer. Using this knowledge, the new model with slow KaiB bonding was altered to include the structural interactions of KaiB:

$$T \xrightarrow{K_{Tdi}} B \qquad (9)$$

Where *T* is one KaiB tetramer, K_{Tdi} is the rate at which the tetrameric form breaks down into a dimeric form, and *B* is 2 KaiB dimers capable of interacting with KaiC. This does not change the actual model of KaiC, it simply adjusts the available amount of KaiB at any given time. If we assume that this interaction also obeys simple mass-action kinetics. Then we know that

$$\frac{d[T]}{dt} = k_{Tdib}[B] - k_{Tdif}[T] \qquad (10)$$

and

$$[B]_t = 2[T] + [B] + 2\sum_{n=0}^{6}[BC^n] + [ABC^n] \qquad (11)$$

where $[B]_t$ is the total KaiB dimers, which is known, [*B*] is the free KaiB dimer, and k_{Tdif} and k_{Tdib} are the rates of forward and reverse dimerization. Solving for the concentration of [*B*] numerically becomes

trivial once this is input into the computational solution of the updated MATLAB model with slow KaiB binding. It was once again a matter of finding a parameter set which gives oscillations.

Results and Discussion

Finding the parameter set which was capable of causing oscillations was far simpler for this problem. As the only thing changed from the previous attempt is the effective KaiB concentration, it makes sense that the only value that needed to change was the binding rate of KaiB. Once again the ratio $\widehat{K}_B^n$ remained identical to the slow binding rate, the only difference was that both the binding and unbinding rates were increased by a factor of 40 to make the binding a rate a constant $4um^{-2}hr^{-1}$ and adjusting the unbinding rate accordingly.

It was discovered that by having an active structural interaction between the dimer and tetramer of KaiB, there was a buffering effect, albeit slight. Figure 8 shows that the effects of additional KaiB are slightly lessened as it takes more than doubling the concentration of KaiB to cause a 20% change in the period of the oscillation. It also shows that the model doesn't "break" till the concentration is over 6.5[KaiA]. However, even with this buffering effect, the reliance on KaiB concentration is still far too high to be the correct model for the mechanism of the system.

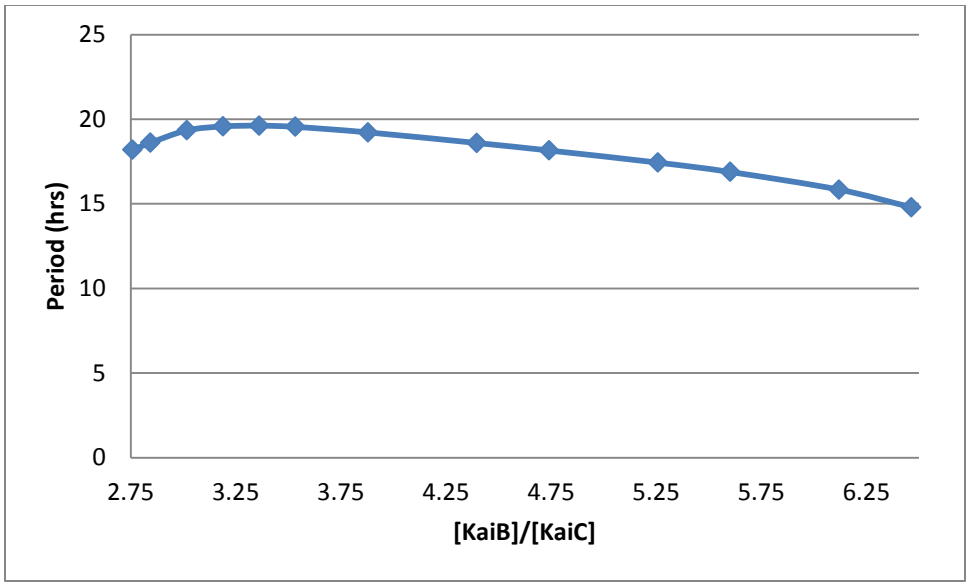

Figure 8: Period dependence on normalized [KaiB]. Again, the dependence on [KaiB] should be negligible above 3[KaiA]. Data taken in MATLAB and plotted in Microsoft Excel.

Conclusion

In this project the mechanism for the *in vitro* oscillations of the ratio of phosphorylated KaiC in the cyanobacterium *S. elongates* was explored. Two possible models were used to attempt to reproduce the wealth of experimental data published about this unusual phenomenon. Unfortunately, according to experimental data done by the Kondo group[16] neither of these models was capable of reproducing the data as both had too strong of a dependence on the concentration of KaiB protein in solution above what is known to be a saturation point. From this project, it is now known that using a very basic model grounded in simplistic mass-action kinetics is unlikely the mechanism for the in vitro circadian rhythm. In the future, a new mechanism will have to be hypothesized. The most recent article[17] (as of early fall 2012) proposes that the mechanism for the interaction is heavily built around the electrostatic interactions of the various conformations of both the dimer and tetramer form of KaiB. As this is a far more complicated mechanism, but still one well-grounded in biophysics, it would be a reasonable next step to begin exploring and modeling these electrostatic forces as a possible mechanism for the *in vitro* circadian rhythm in the phosphorylation of the KaiC protein in the cyanobacterium *Synechococcus elongatus.*

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