Synchronized Cycles: An allosteric model of the Kai circadian oscillator

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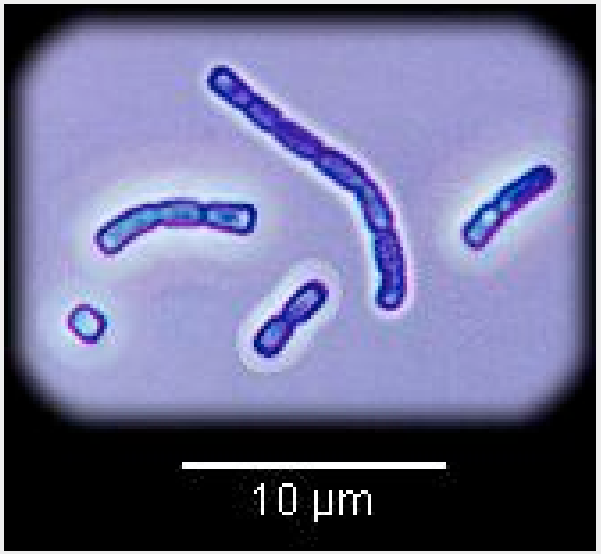
Pim Altena
Pieter Rein ten Wolde
(*AMOLF, Amsterdam*)

S. elongatus

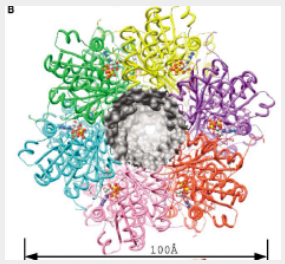


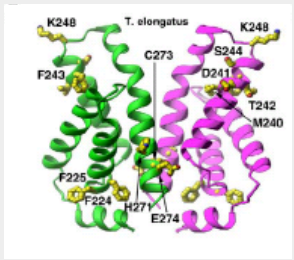
(Image:CIBNOR)

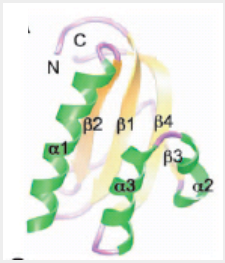
PNAS **104**, 7420 (2007)

The Problem…

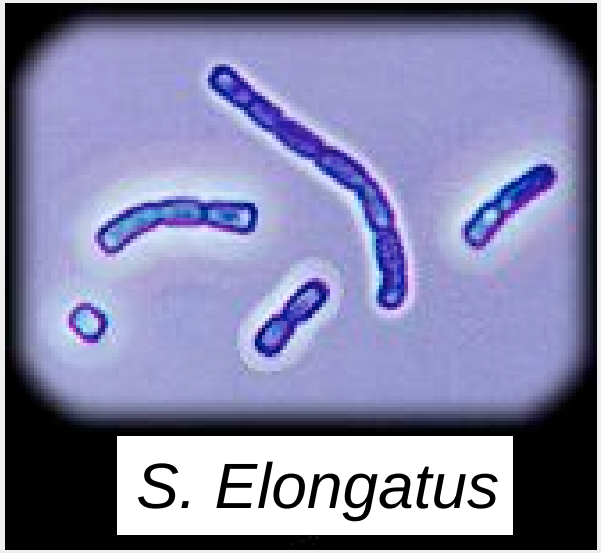
- Circadian rhythm in a test tube:

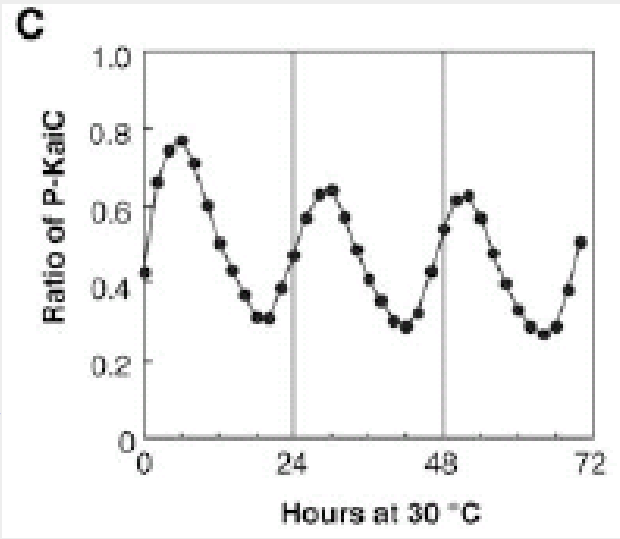






3 proteins (+ ATP)





Oscillatory phospho-rylation

(Nakajima *et al.*, *Science* 2005)

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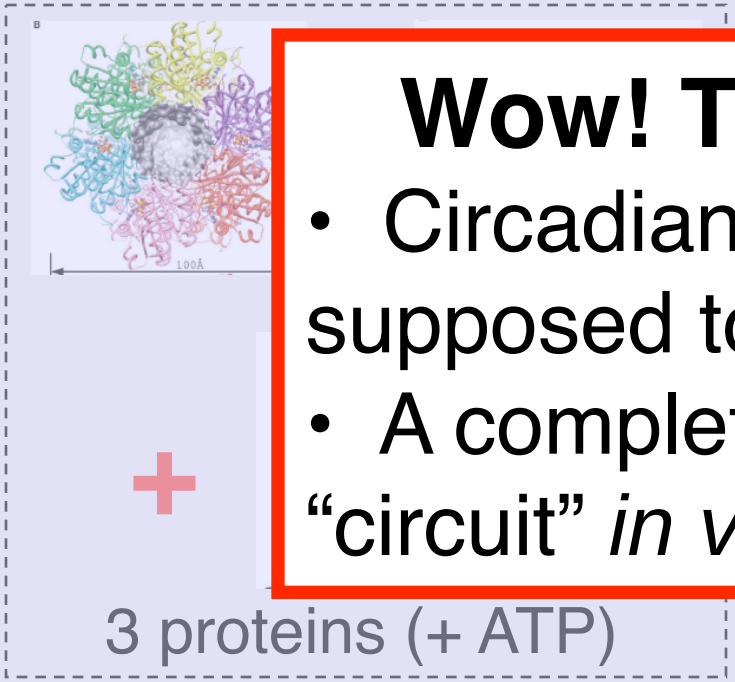

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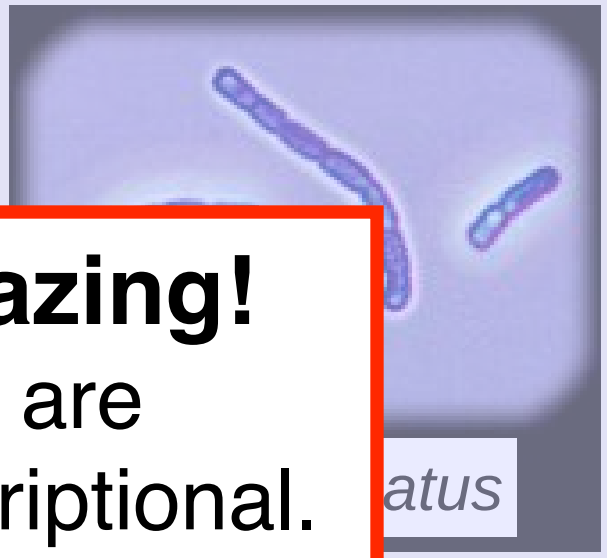
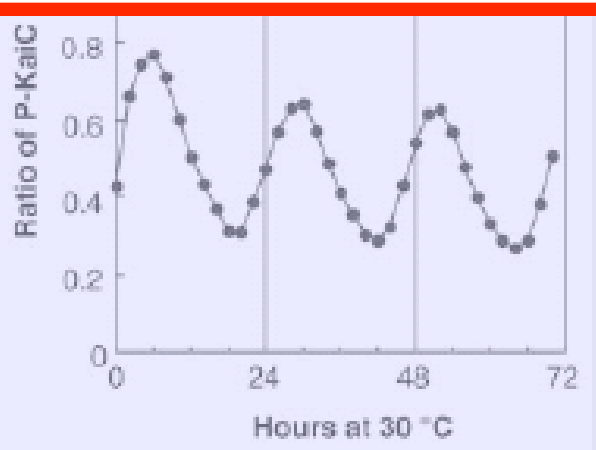


Wow! That's amazing!

- Circadian oscillators are supposed to be transcriptional.
- A complete biochemical "circuit" *in vitro*.



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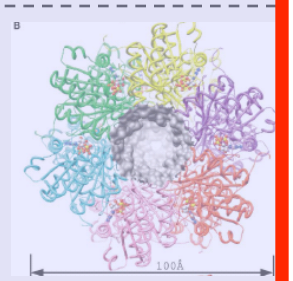
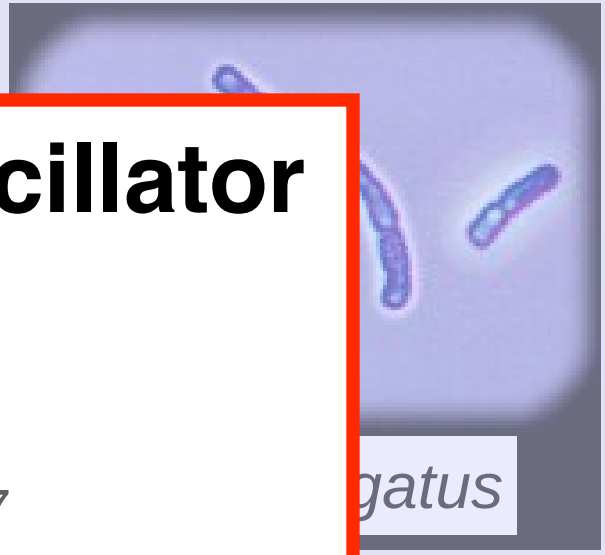




gatus

+

3 prote

scillatory
hospho-
lation
akajima *et al.*, *Science* 2005)

How does this oscillator work?

Related models:
Emberly and Wingreen, *PRL* 2006
Clodong *et al.*, *Mol. Sys. Biol.* 2007
Mori *et al.*, *PLoS Biol.* 2007
Yoda *et al.*, *PLoS ONE* 2007

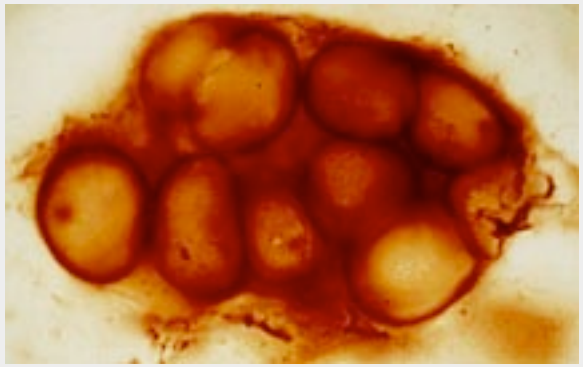
Other models:
Mehra *et al.*, PLoS Comp. Biol. 2006
Miyoshi *et al.*, JBR 2007
Takigawa-Imamura and Mochizuki, J. Th. Biol. 2006
" " JBR 2006

Outline

- Background
  - Cyanobacteria
  - *In vitro* data
- Allosteric Cycles: Focus on hexamers
  - Thermodynamic consistency
- Synchronization by Differential Affinity
  - Alternative proposals
- Full Model
  - Matching available data
  - “Temperature compensation”
- Conclusions

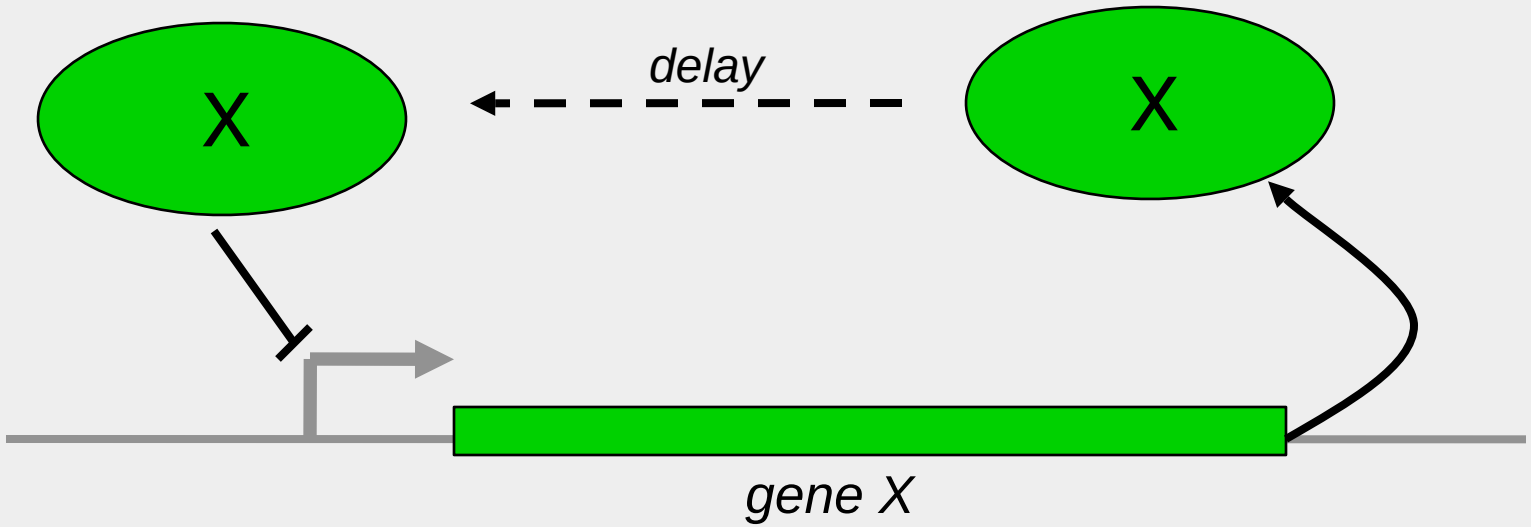
Cyanobacteria

- Photosynthetic bacteria (O_2 producing)
- Oldest known fossils
  - ~3.5 billion years
  - Responsible for current oxygenic atmosphere
  - Origin of chloroplasts
- "Blue-green algae"
- *S. elongatus*: Genetically tractable model.



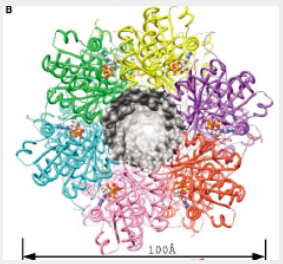
Circadian Rhythms

- Most eukaryotes, cyanobacteria,…
- Free-running oscillation, ~24 hours.
- Entrained by light, temperature, etc.
- Temperature-compensated.
- Textbook model: Negative transcriptional feedback



…but no transcription needed in *S. Elongatus*

- Circadian rhythm in a test tube:



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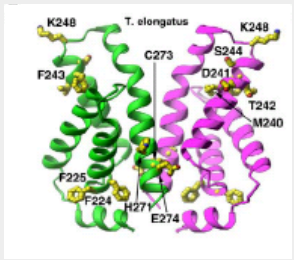

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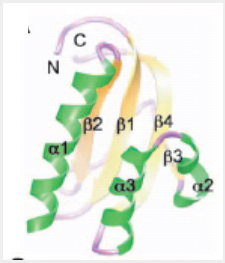

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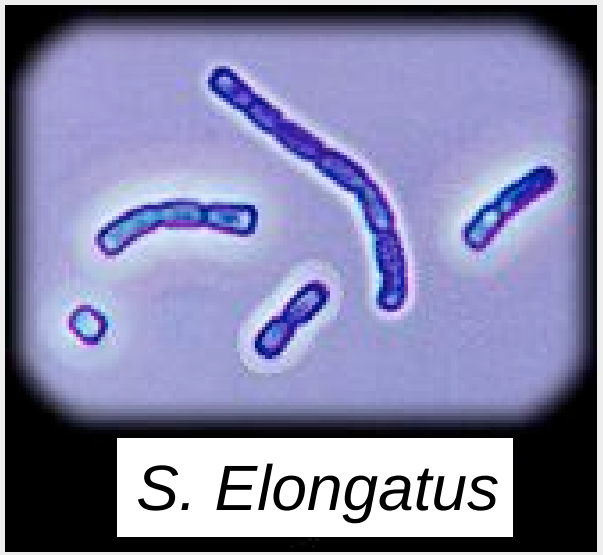

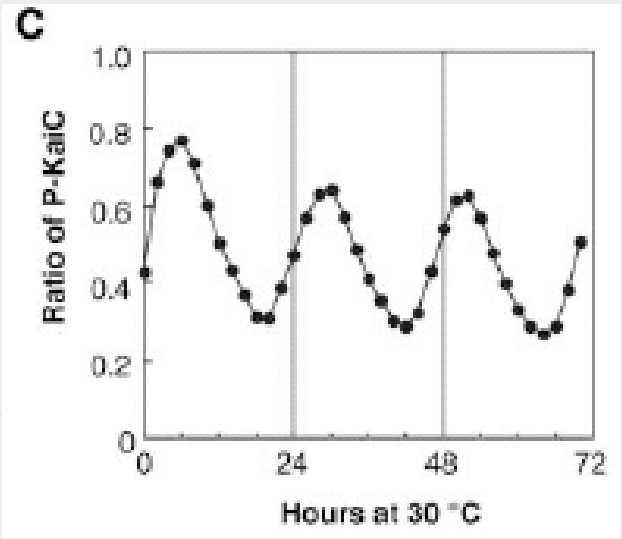

Oscillatory phospho-rylation

(Nakajima *et al.*, *Science* 2005)

The Players

- KaiC
  - Hexamer (AAA+ ATPase).
  - Auto (de)phosphorylation activity.
  - Transcriptional repressor *in vivo*.
  - Phosphorylation level oscillates with 24 hour period.
- KaiA
  - Dimer
  - Stimulates KaiC phosphorylation.
  - Complexes w/KaiC and KaiB + KaiC.
- KaiB
  - Dimer or tetramer
  - Attentuates KaiA's effects.
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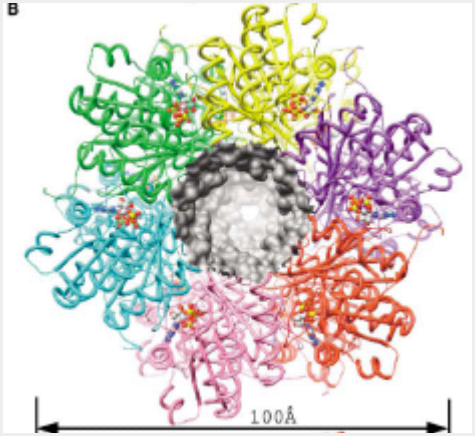

Pattanayek *et al.*, *Mol. Cell* 2004

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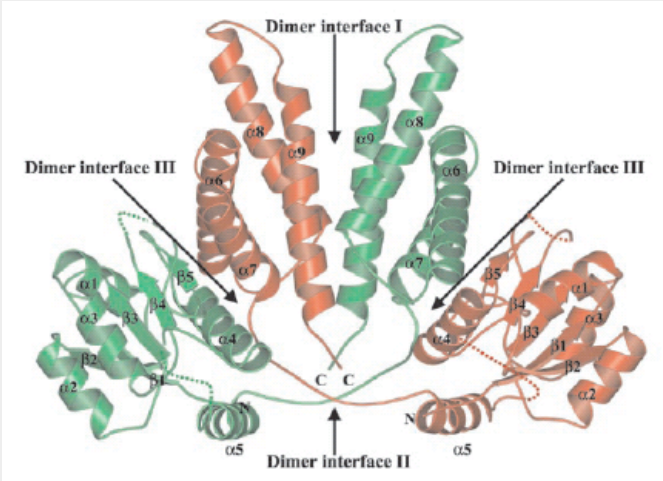

Ye *et al.*, *J Biol. Chem.* 2004

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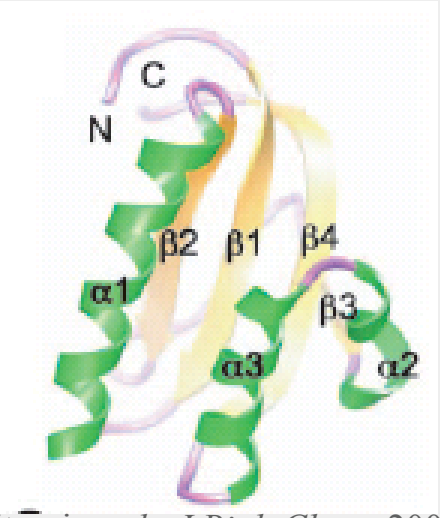


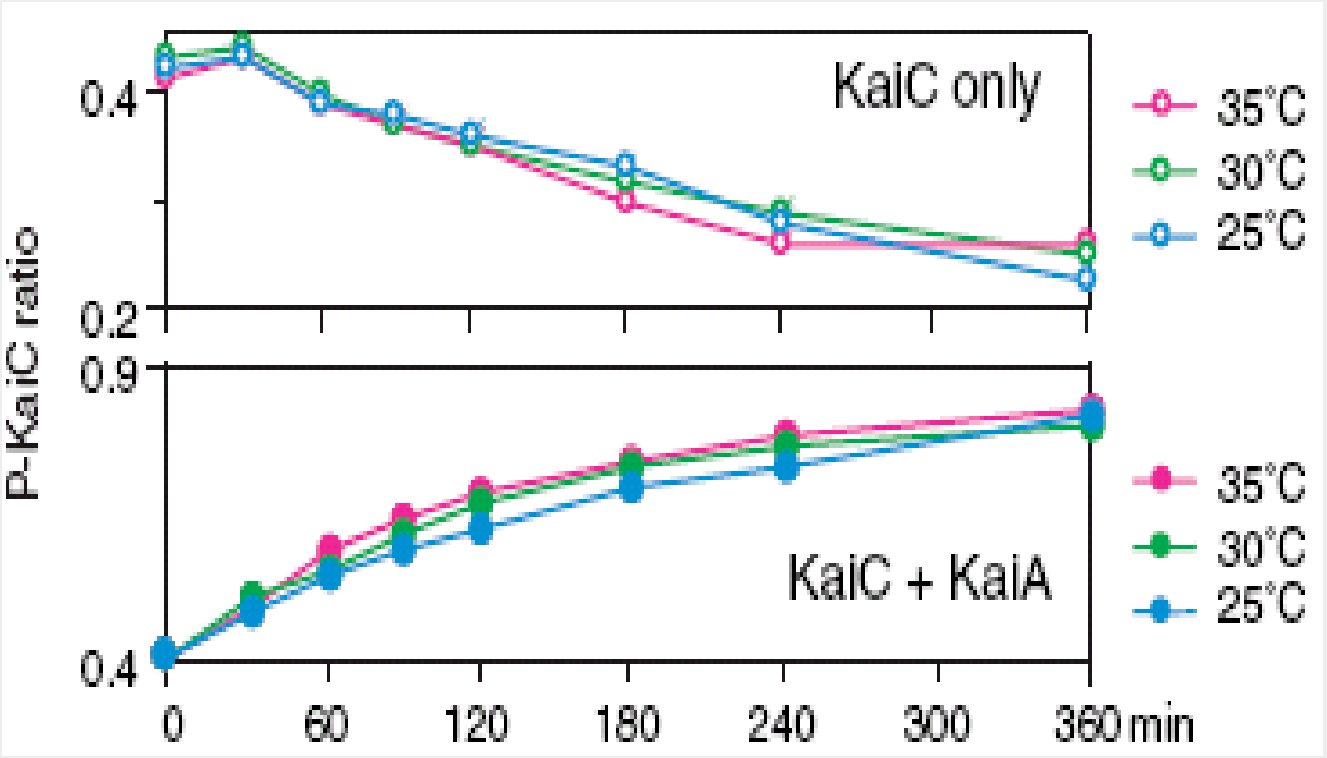
Hitomi *et al.*, *J Biol. Chem.* 2005

In Vitro Data

- Different combinations of proteins
  - Phosphorylation and dephosphorylation each slow and temperature-compensated.

(to 0 at long times)

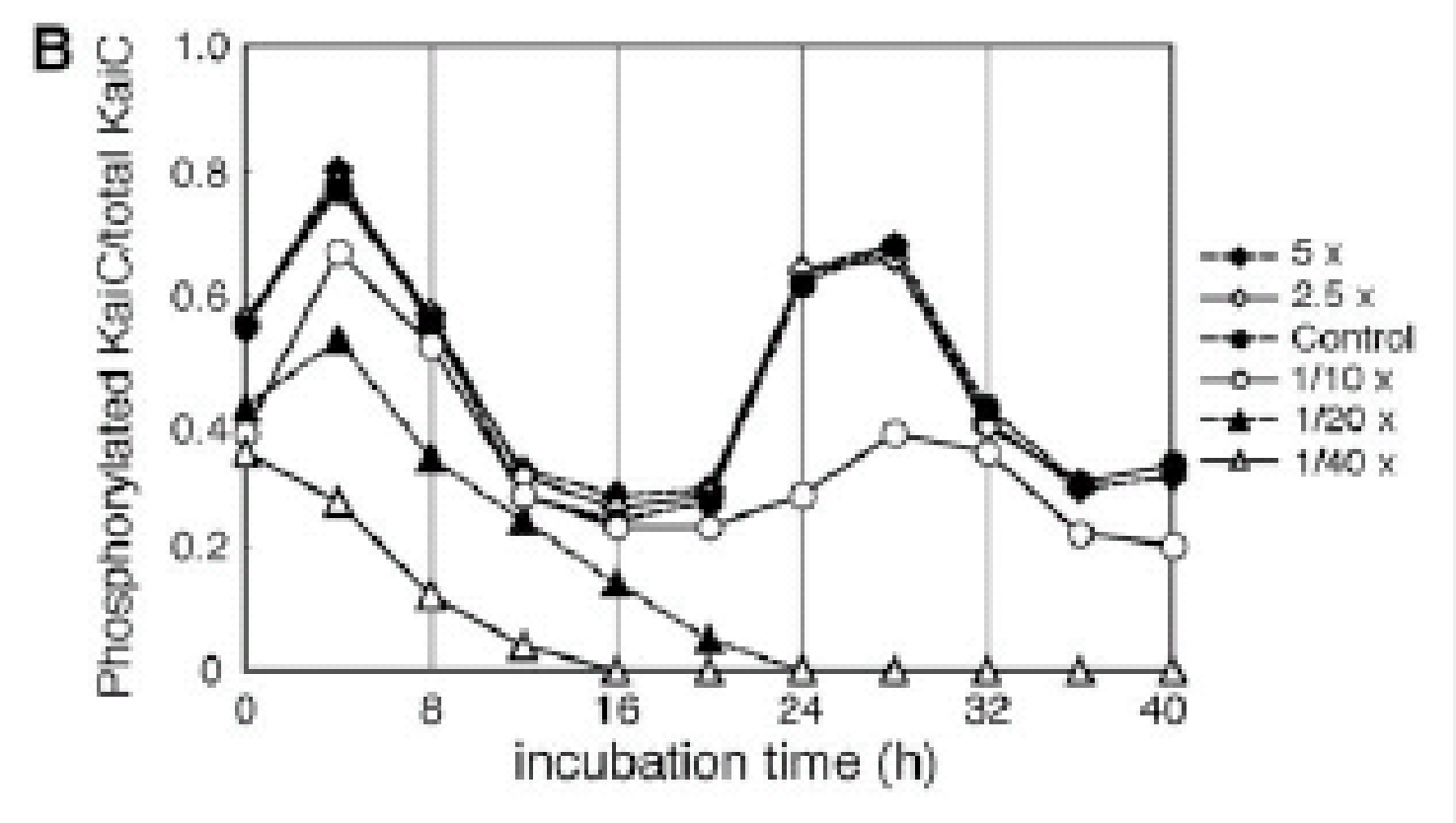
(to 1 at long times)



Tomita *et al.*, *Science* 2005

In Vitro Data

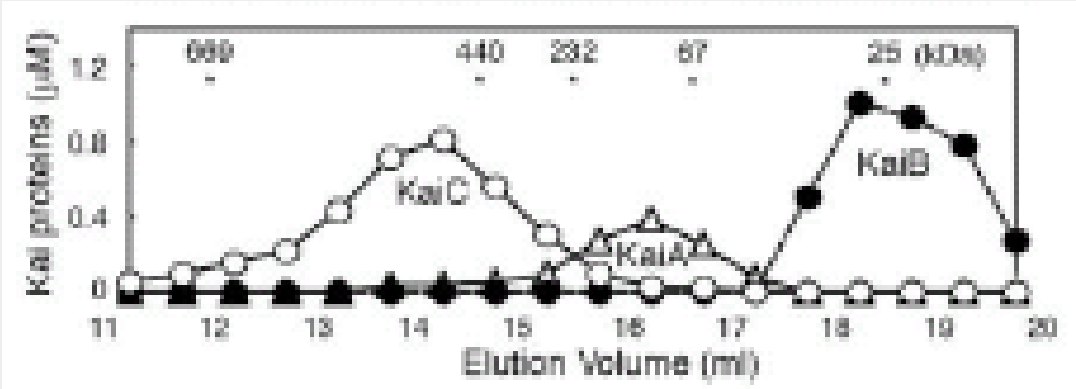
- Varying concentrations
  - Increasing all concentrations by same factor ⇒ No change.



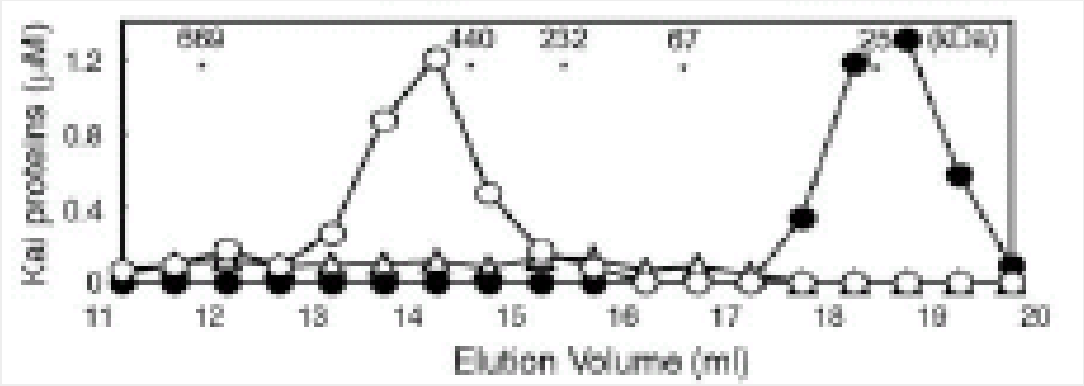
Kageyama *et al.*, *Mol. Cell* 2006

In Vitro Data

- Sizes of Complexes
  - No evidence for interactions between KaiC hexamers.
  - Almost no free KaiA.



t = 0 (reference)



t = 24 hrs.

Kageyama *et al.*, *Mol. Cell* 2006

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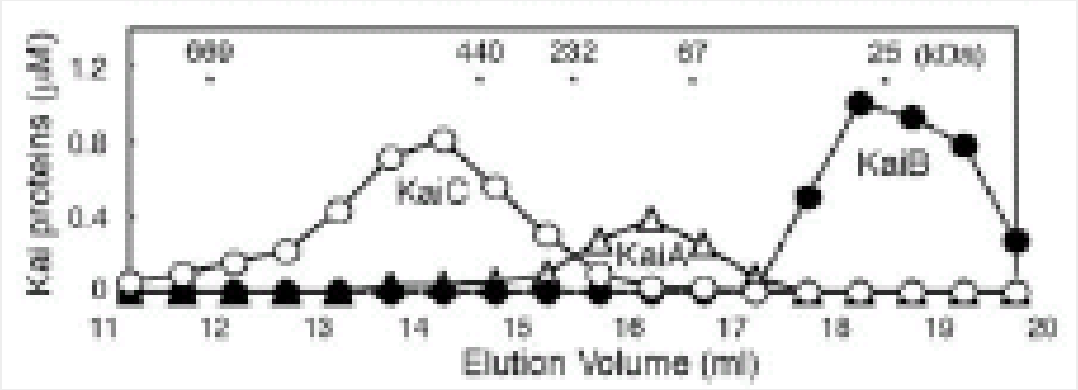

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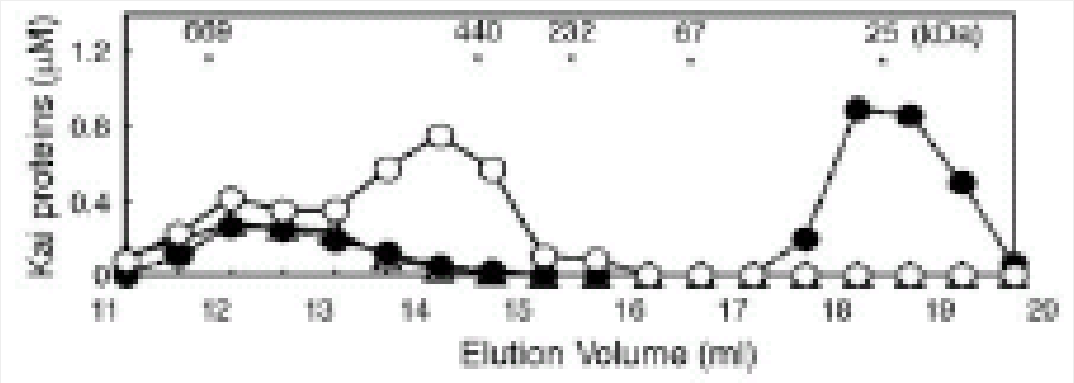
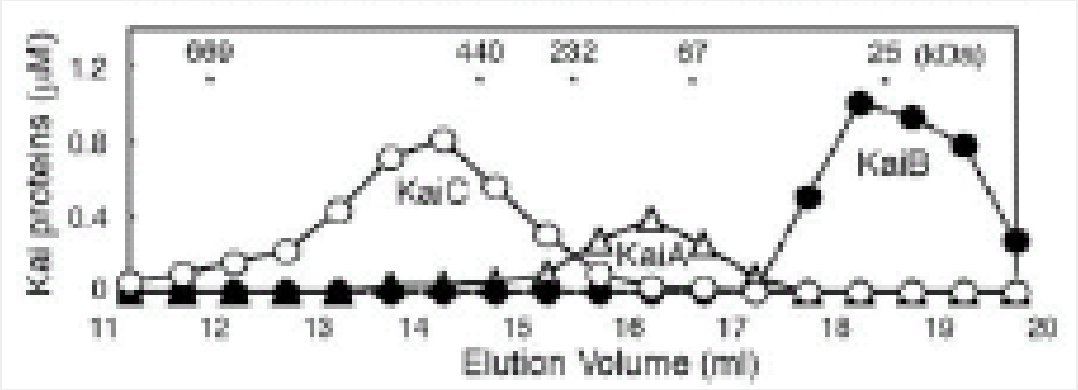


t = 30 hrs.

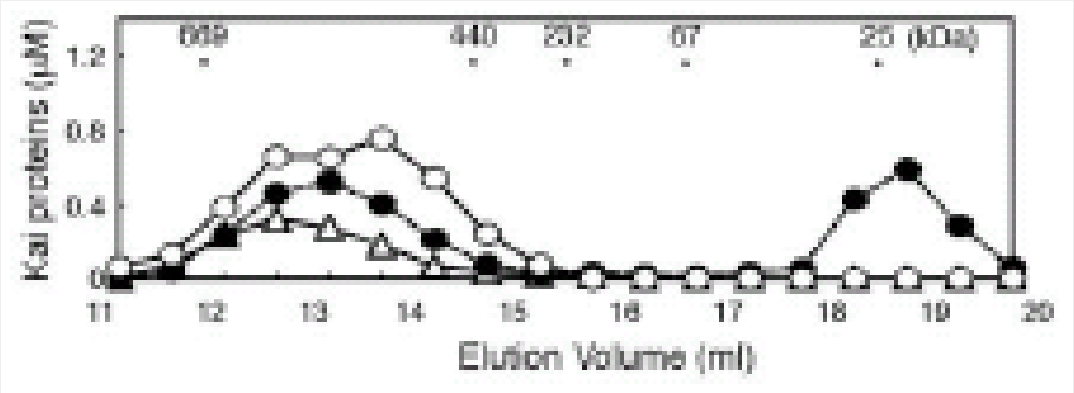
Kageyama *et al.*, *Mol. Cell* 2006

In Vitro Data

- Sizes of Complexes
  - No evidence for interactions between KaiC hexamers.
  - Almost no free KaiA.



t = 0 (reference)



t = 36 hrs.

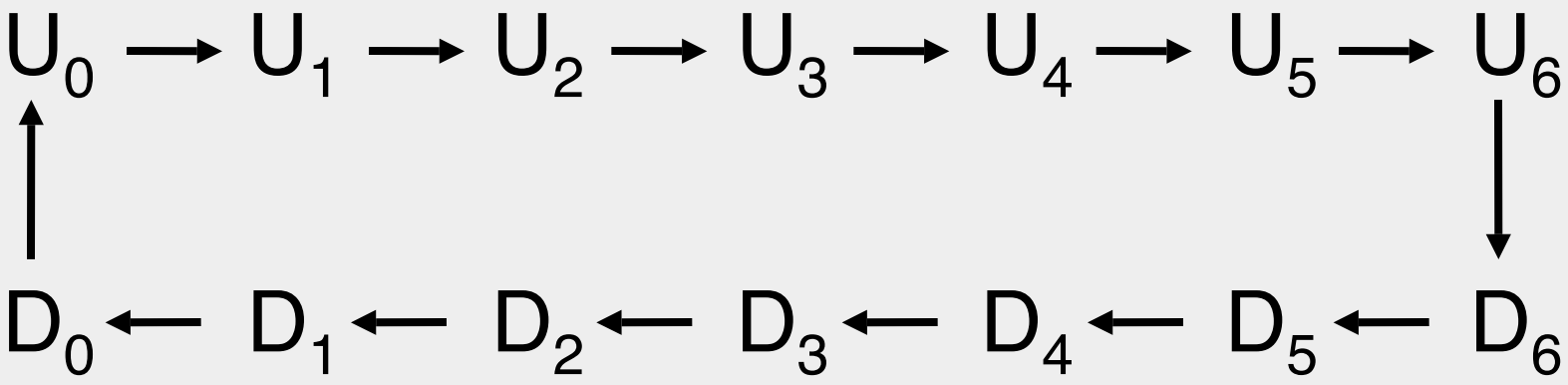
Kageyama *et al.*, *Mol. Cell* 2006

Modeling Challenges

- Kai proteins neither created nor destroyed.
- KaiC (de)phosphorylation = **only** driven (energy-consuming) reactions.
  - No other covalent modifications or enzymatic activities.
  - Other reactions obey detailed balance (unless tightly coupled to phosphorylation cycle).
- KaiC hexamers don't interact directly.
  - Single KaiC hexamer can't oscillate coherently.
  - KaiC's coupled only indirectly through KaiA and KaiB.
- Seriously constrained by biochemical data.

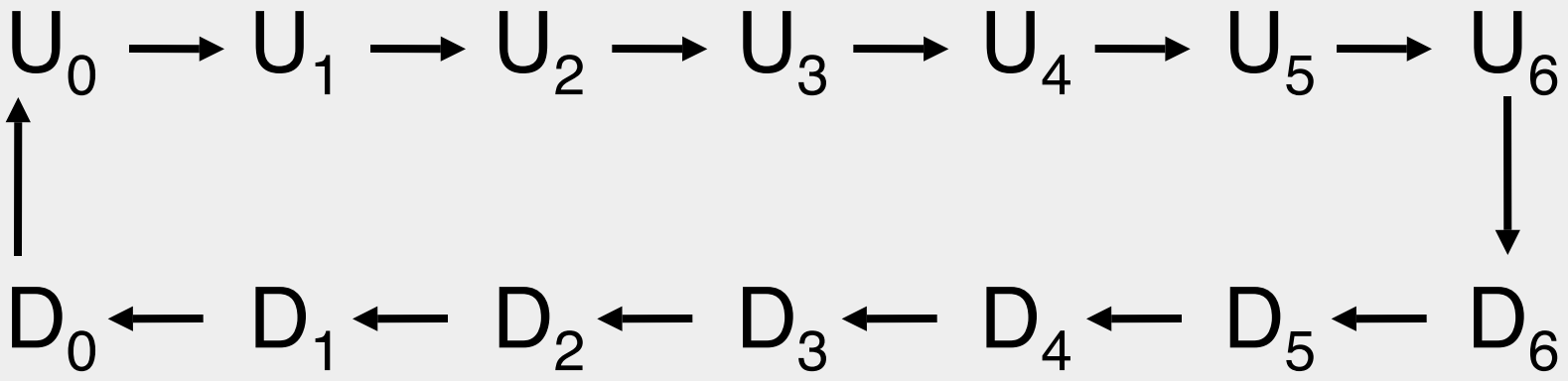
Model, Part 1: Allosteric Cycles

- 2 KaiC conformations:
  - "U" favors phosphorylation
  - "D" favors dephosphorylation
- KaiC alone cyclically adds & removes P's:



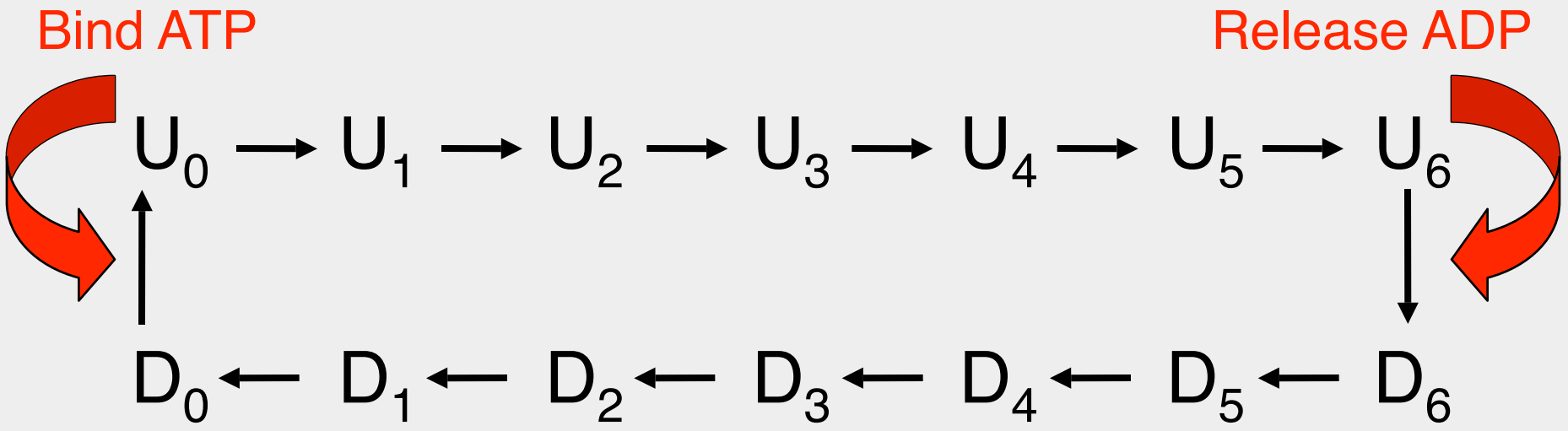
(subscript indicates # phosphates)

Thermodynamic Constraints



Thermodynamic Constraints

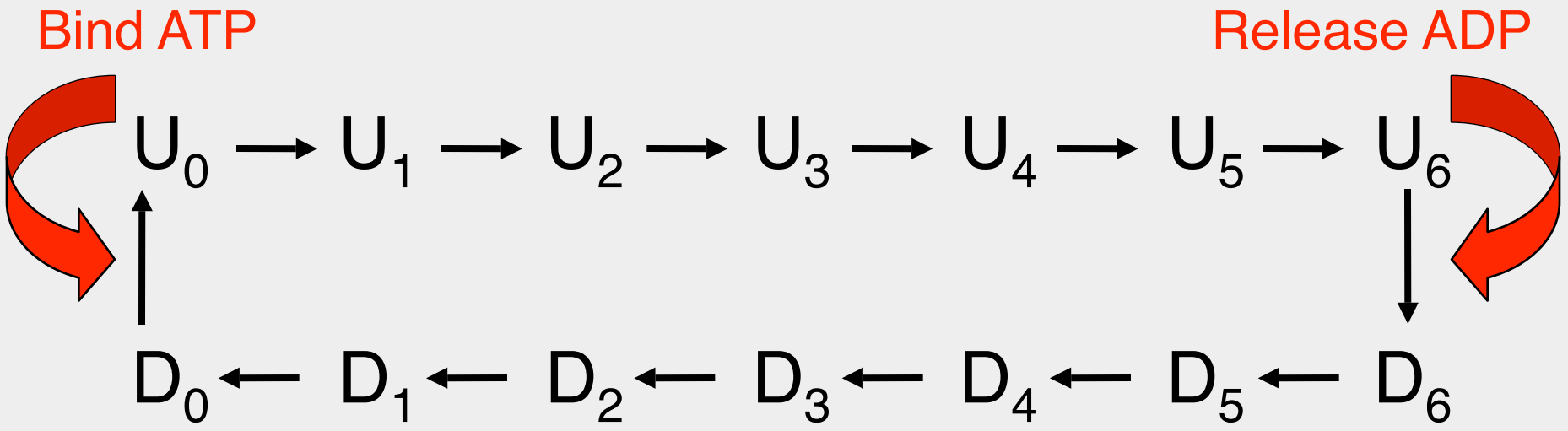
- What drives state transitions?



Thermodynamic Constraints

- What drives state transitions?

  Nucleotide exchange.



Thermodynamic Constraints

- What drives state transitions?

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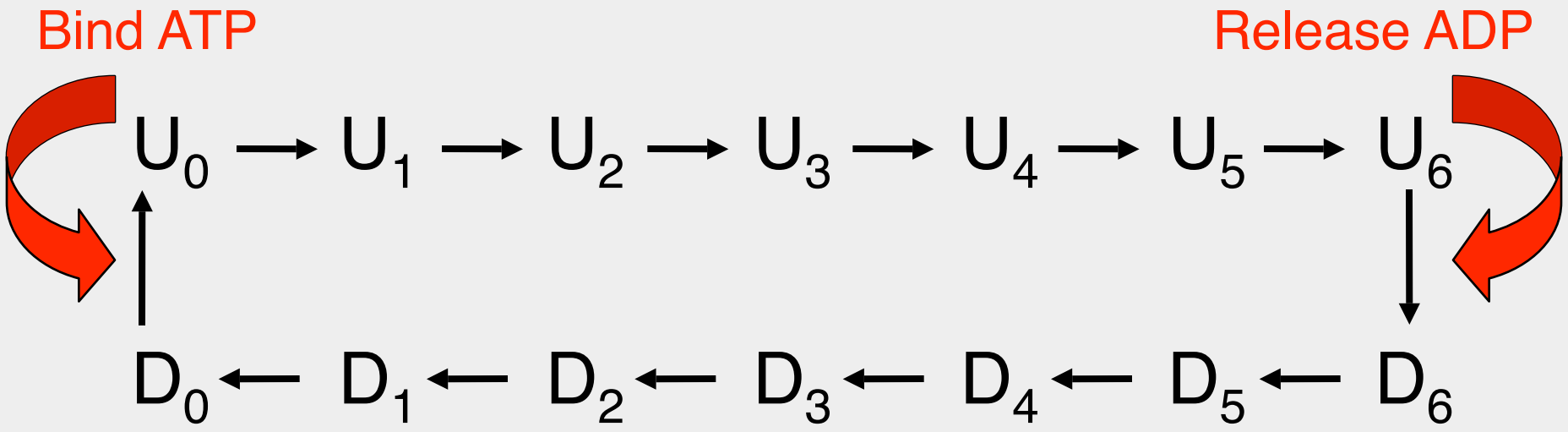

Thermodynamic Constraints

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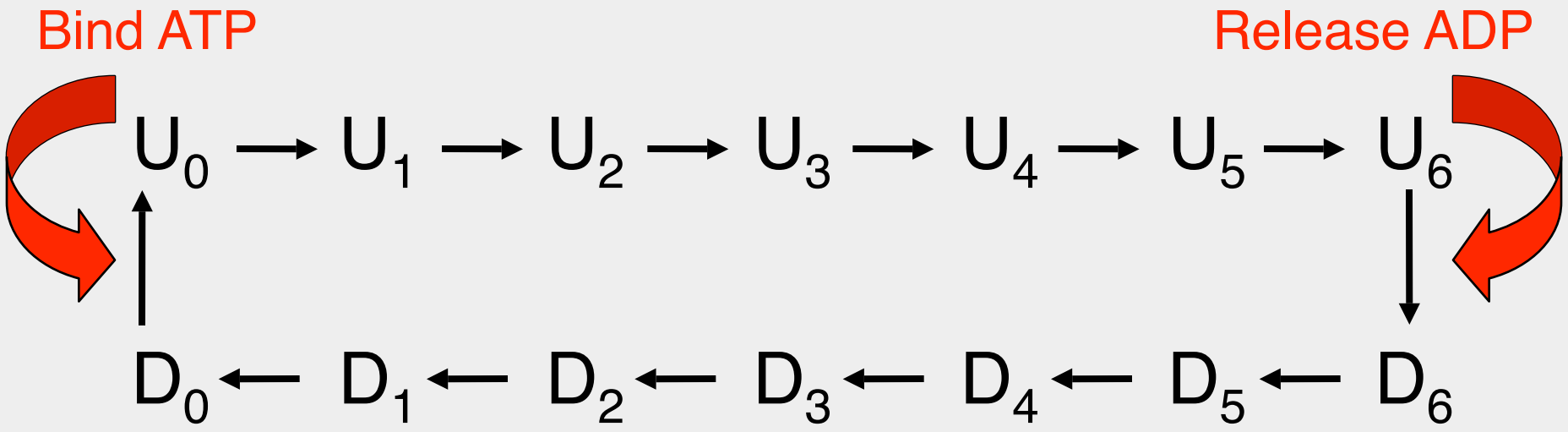
  - All obvious driven steps now “used up”.
  - Further reactions should obey detailed balance.



Thermodynamic Constraints

- What drives state transitions?

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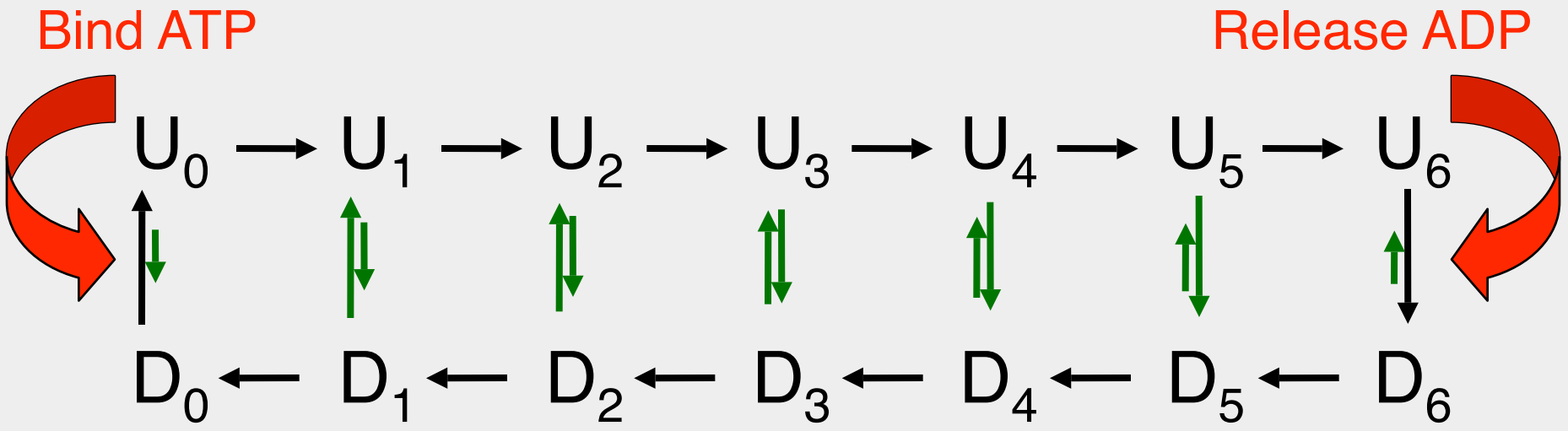
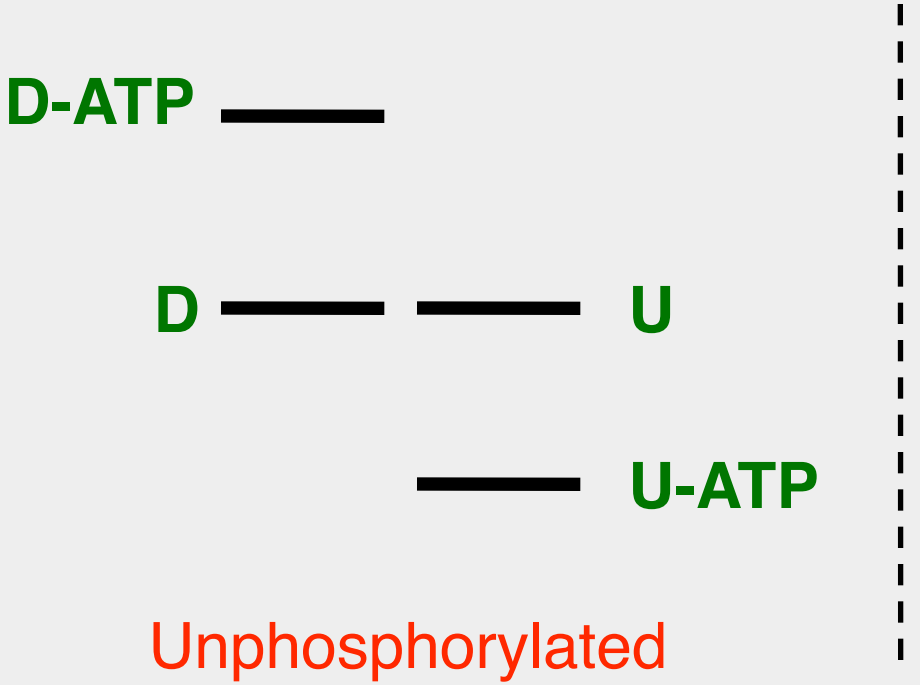
  - All obvious driven steps now “used up”.
  - Further reactions should obey detailed balance.
- Must allow reverse, intermediate reactions.



Monomer States

- 8 KaiC monomer states:
  - U or D conformation
  - Phosphorylated or not
  - Nucleotide (ATP/ADP) bound or not
- Energy levels



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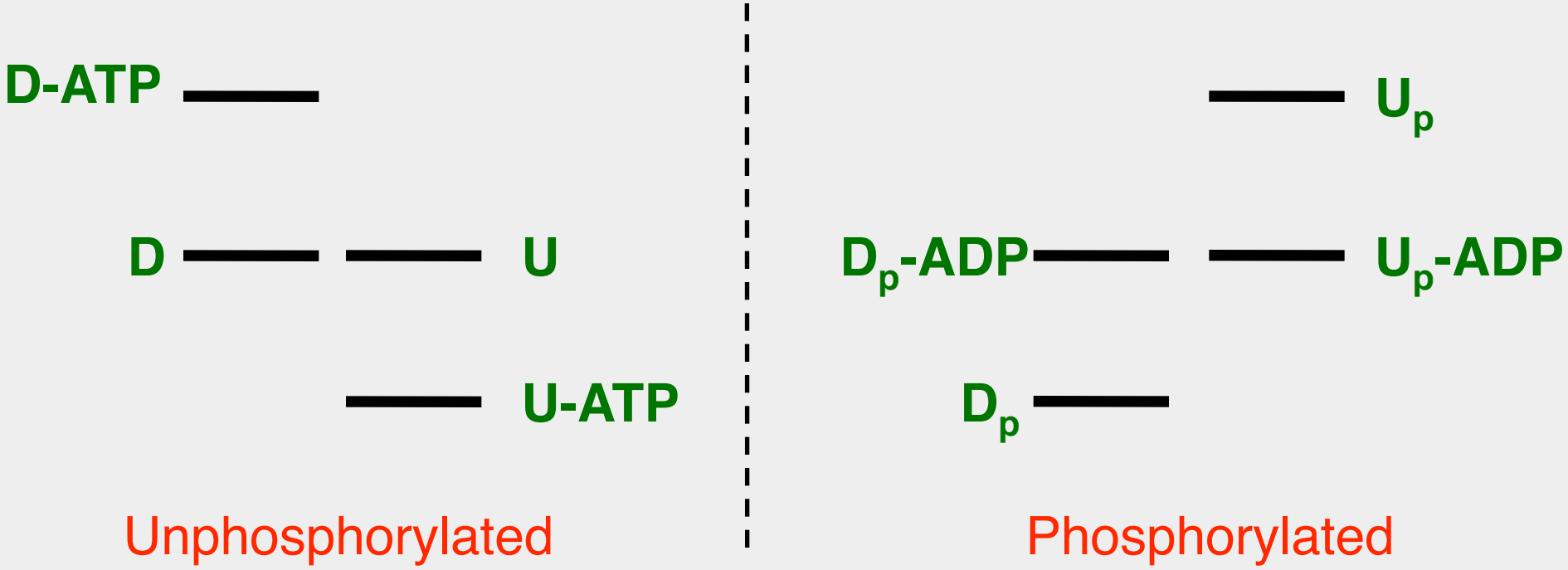
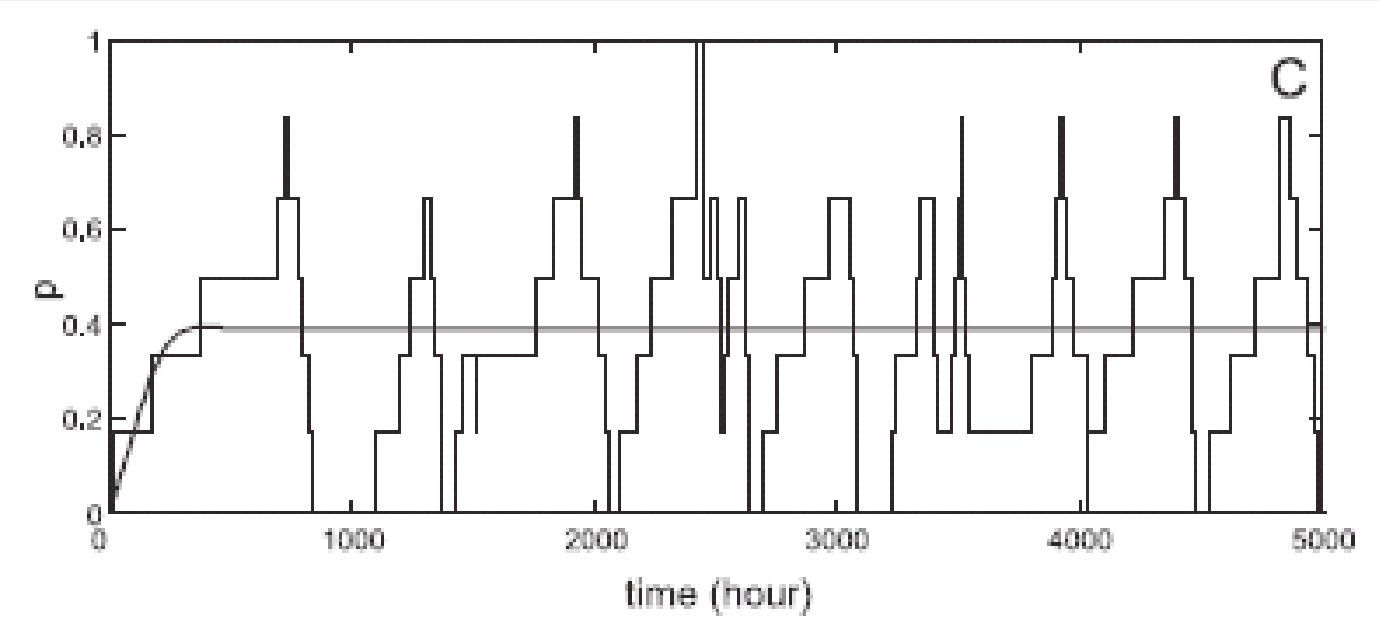


Single Hexamer: Noisy Oscillations

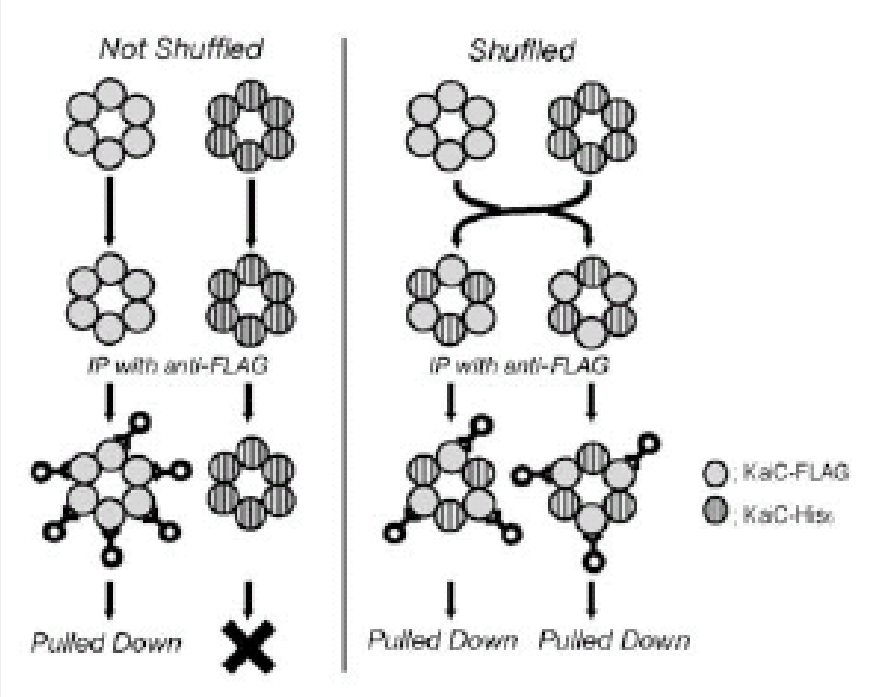


Must **synchronize** the different hexamers.

Monomer Exchange

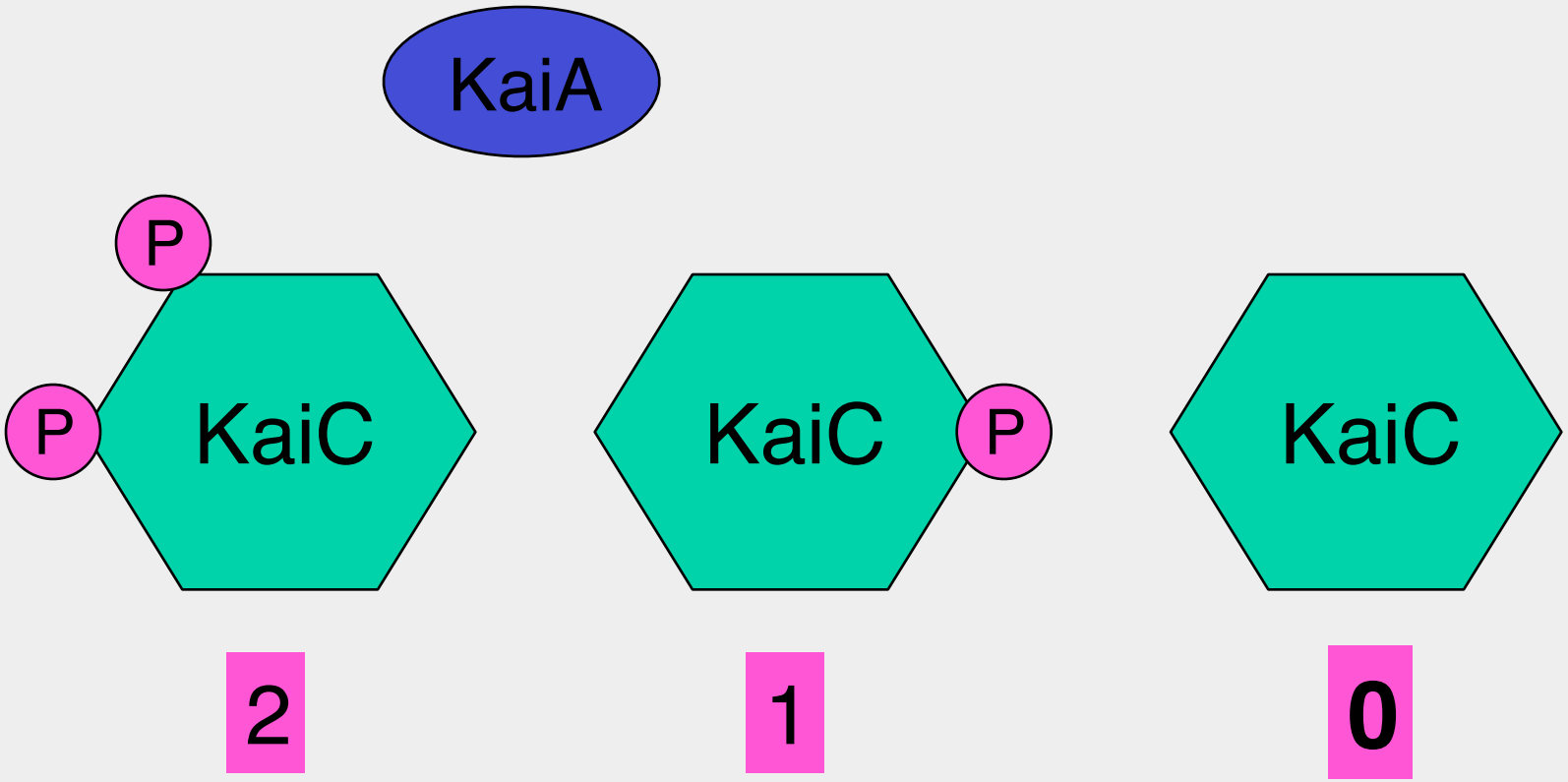
Emberly & Wingreen
PRL 2006

- It happens during dephosphorylation phase.
- It is (probably) not enough to explain synchronization



Model, Part 2: Differential Affinity

- KaiA catalyzes KaiC phosphorylation.
- [KaiA] limiting.
- KaiA binds laggards (fewer phosphates) more strongly than leaders (more phosphates).



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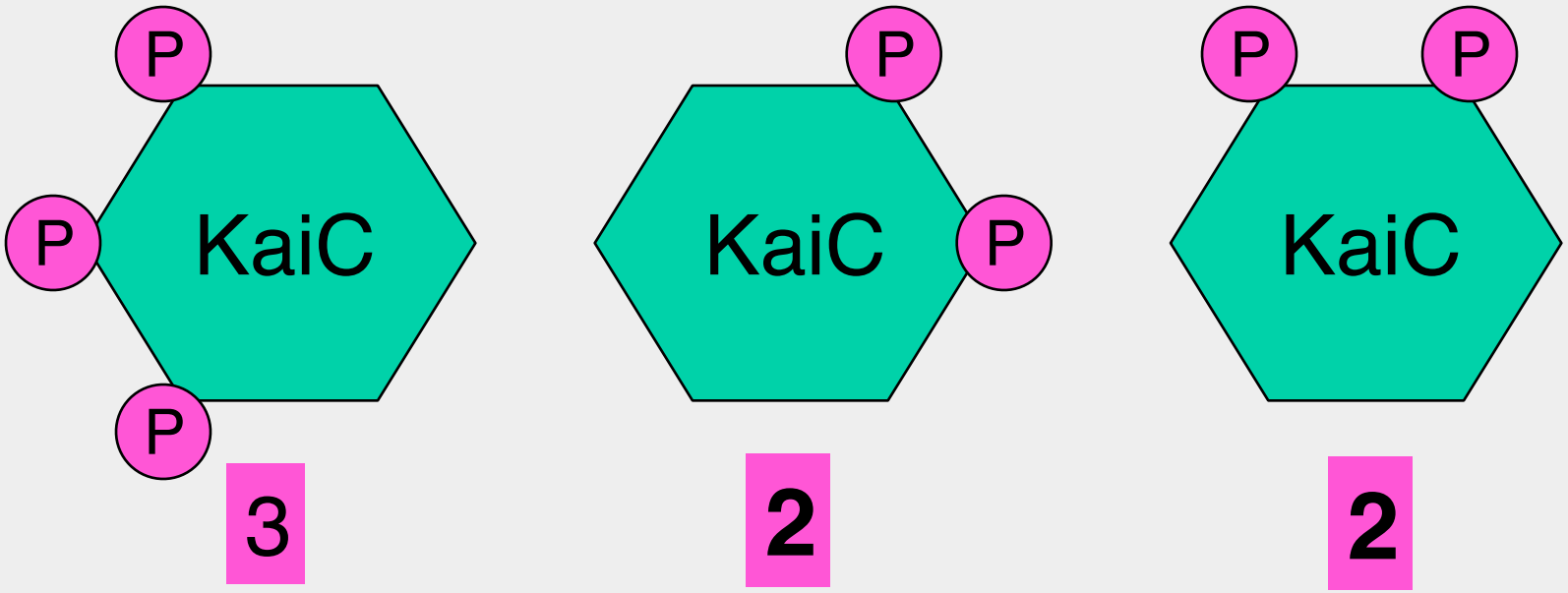
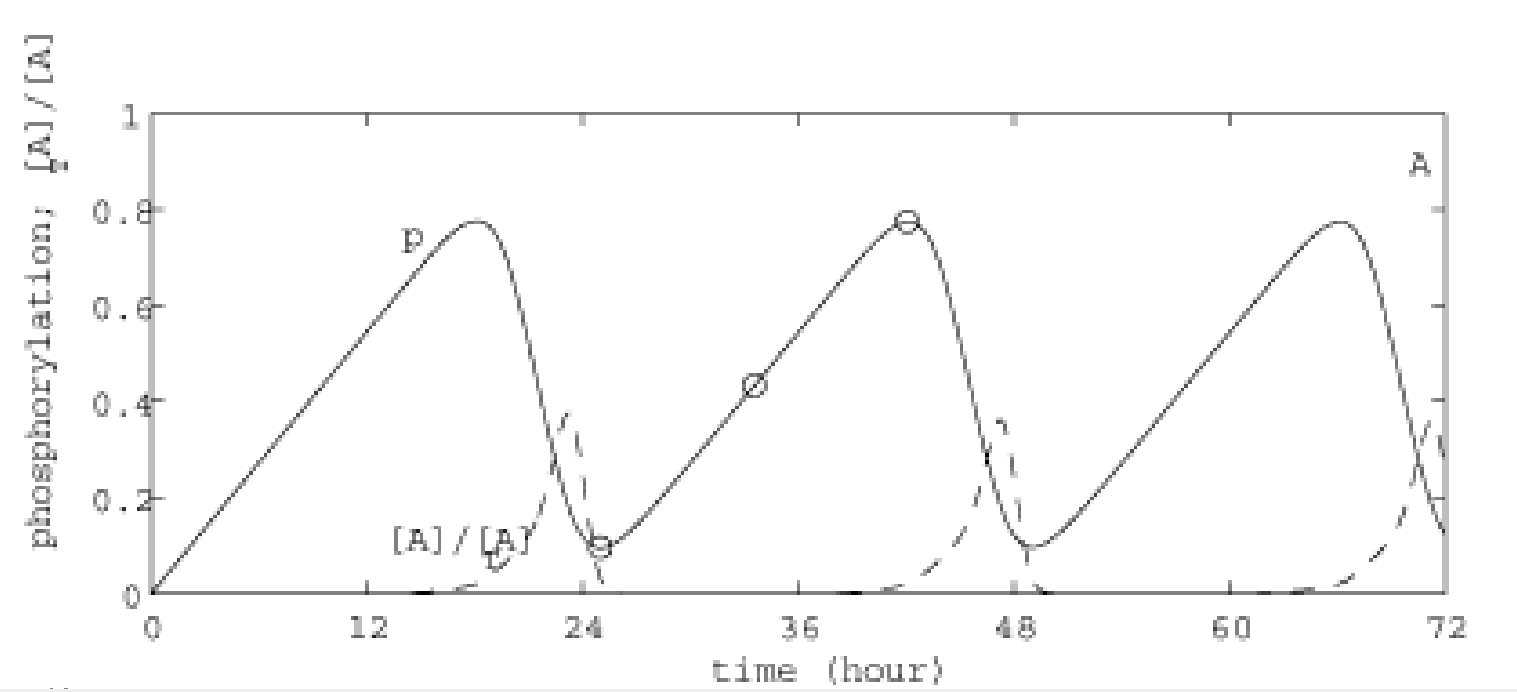


Generic Differential Affinity

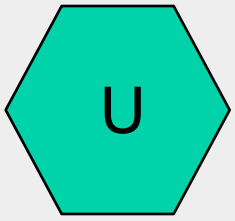
- KaiA and KaiC only.
- Oscillates:



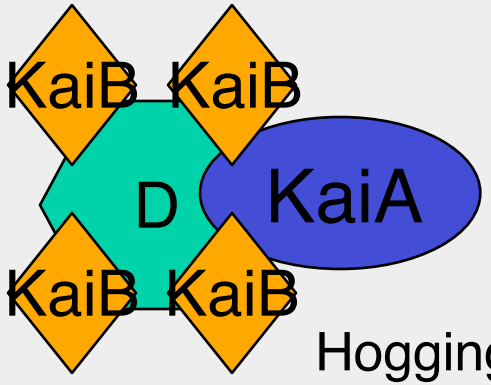
- Does not agree quantitatively with experiments.

Full Model

- Should include KaiB.
- Should agree with data on KaiC alone, KaiA + KaiC, abundance of different complexes,…
- Changes/Additions:
  - Weak dephosphorylation in U conformation.
  - U more stable than D: AVOID OVERSHOOTS.
  - KaiB binds to, stabilizes D conformation.
  - KaiB-KaiC complexes sequester KaiA (differential affinity).

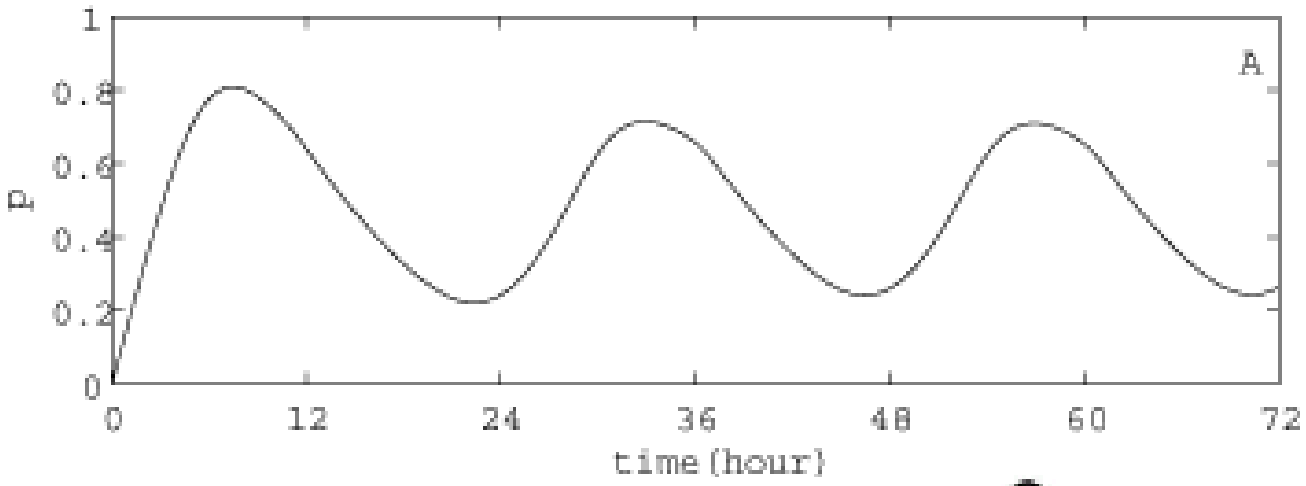


No KaiA, can't add P's



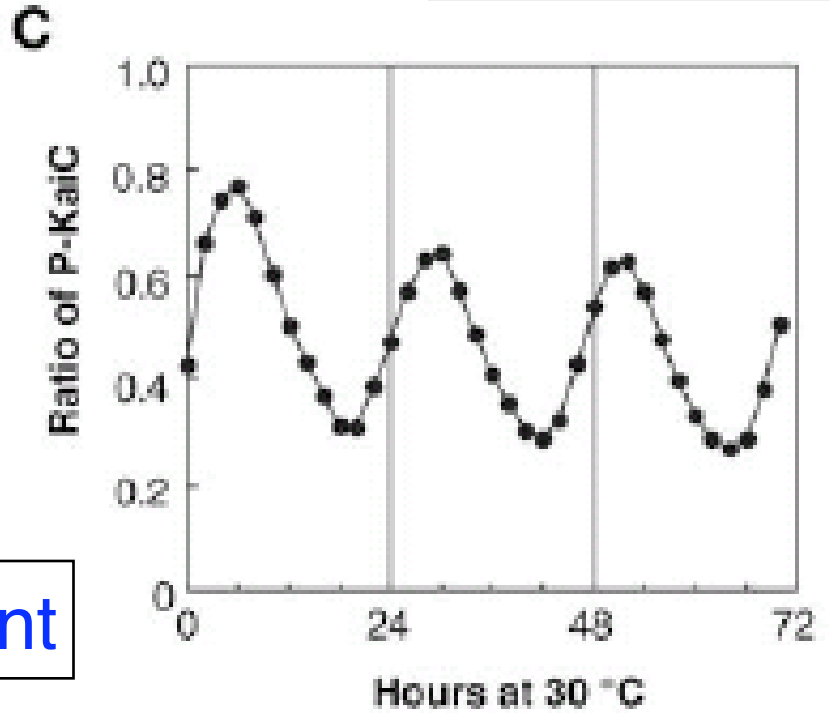
Hogging KaiA

Full Model vs. Experiment



Model

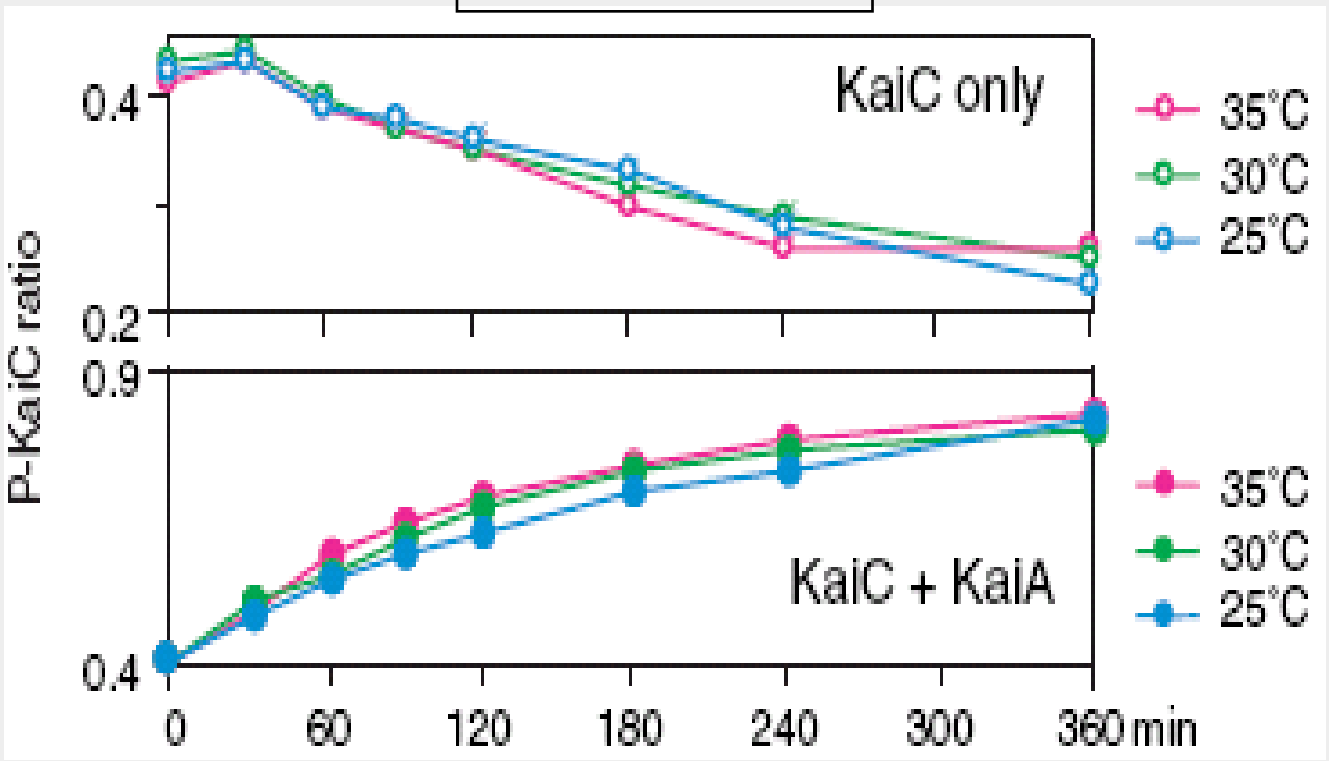
Oscillations



Experiment

Full Model vs. Experiment

Experiment

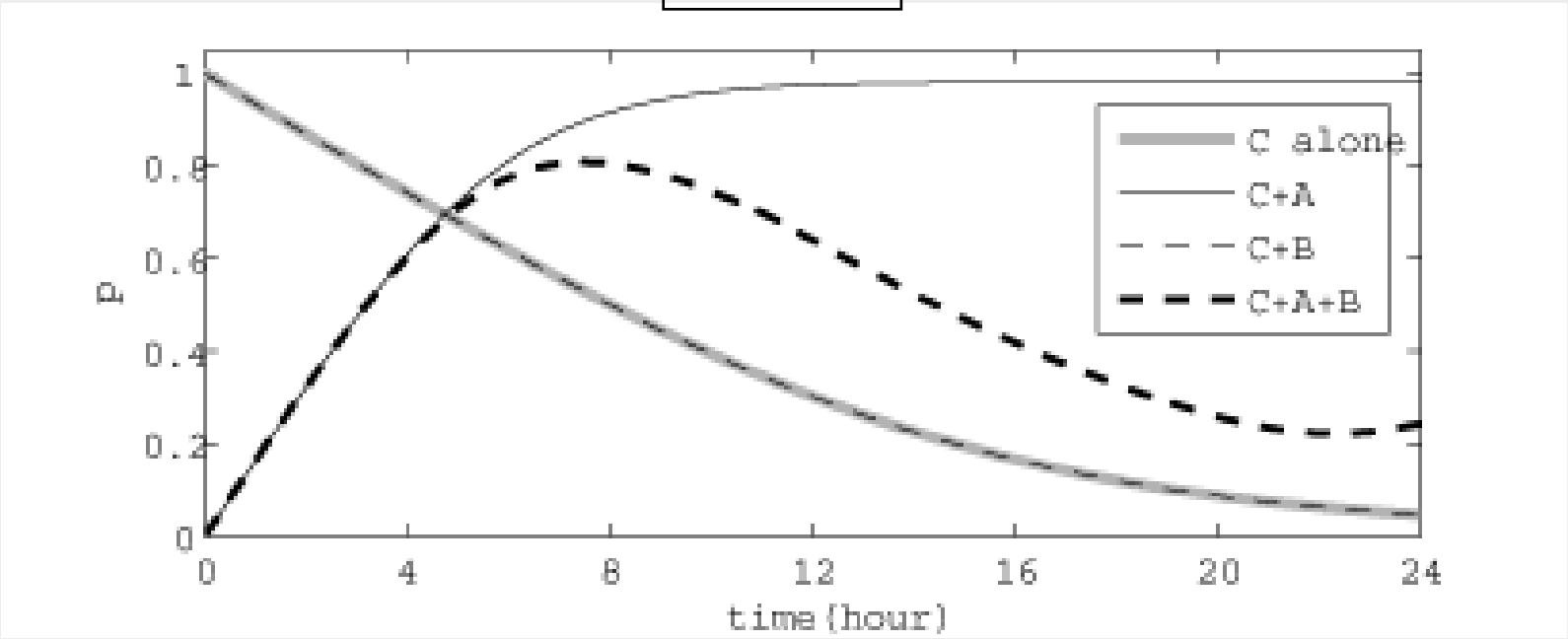


Tomita *et al.*, *Science* 2005

1 or 2 Kai proteins

Full Model vs. Experiment

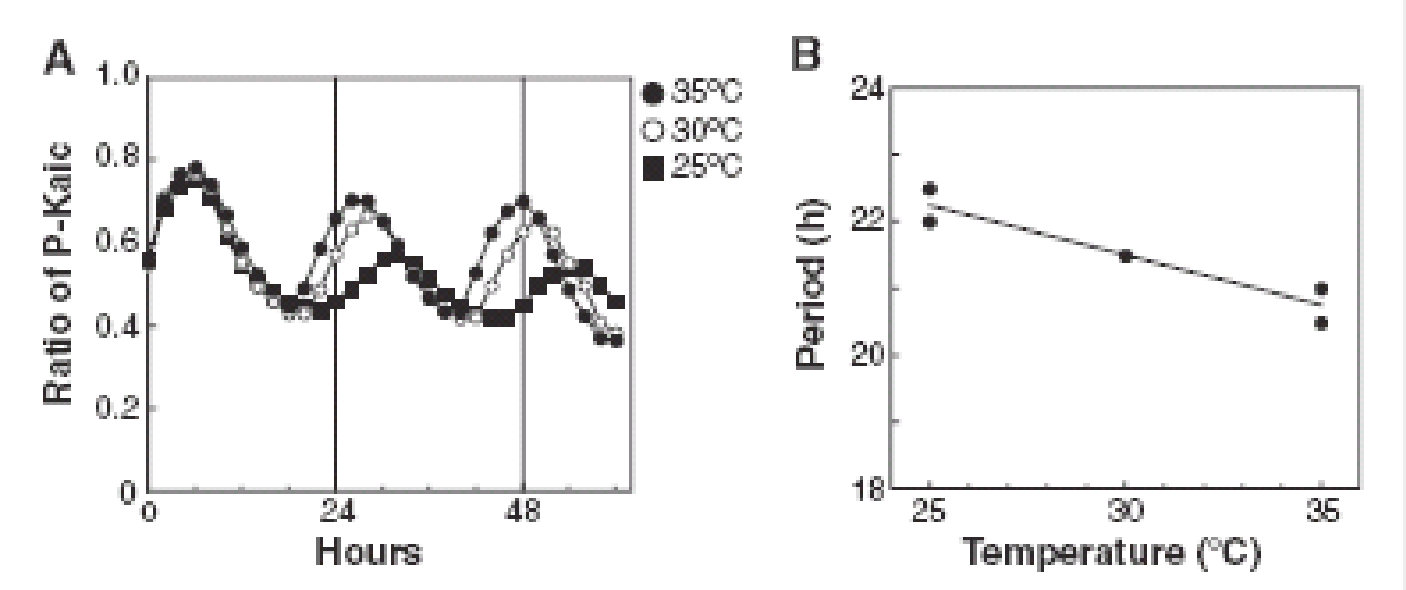
Model



1 or 2 Kai proteins

Temperature Compensation

- Recall
  - Period insensitive to temperature.
  - (De)phosphorylation rates separately insensitive to temperature.



Nakajima *et al.*, *Science* 2005

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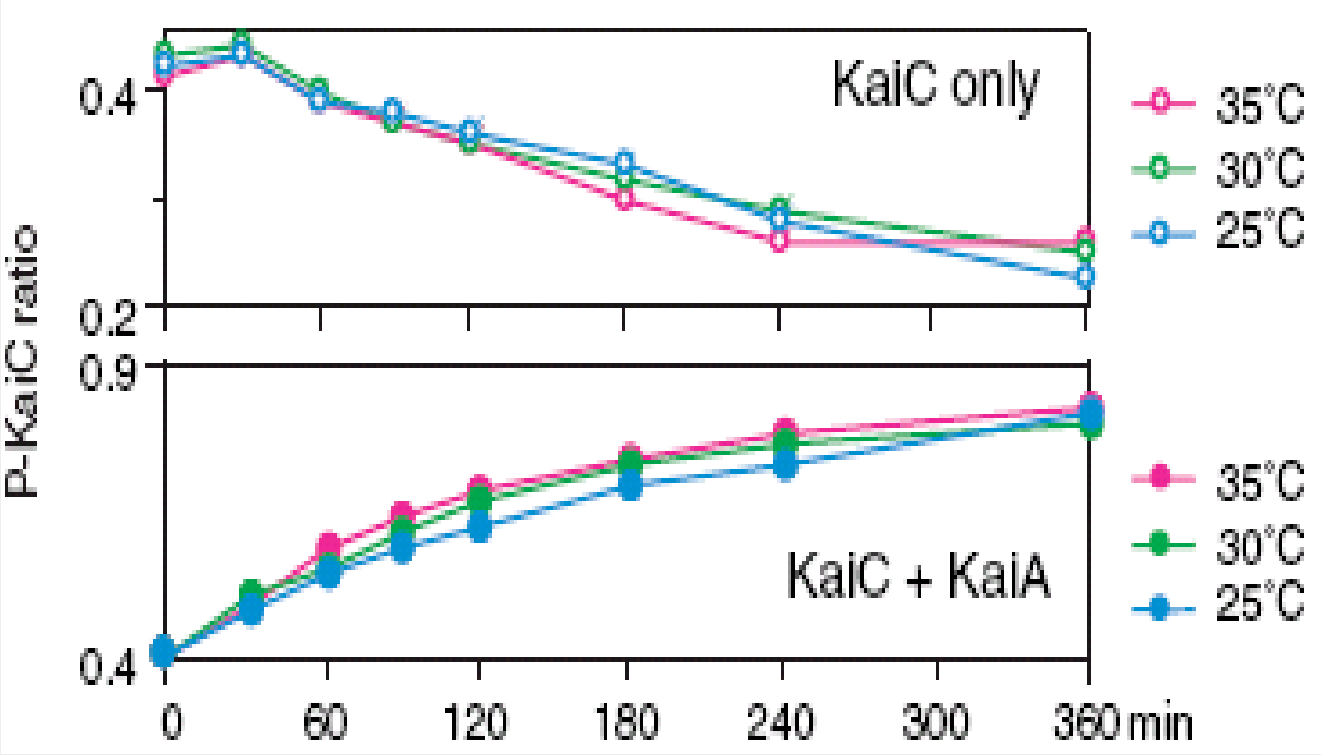

Tomita *et al.*, *Science* 2005

T.C. Model

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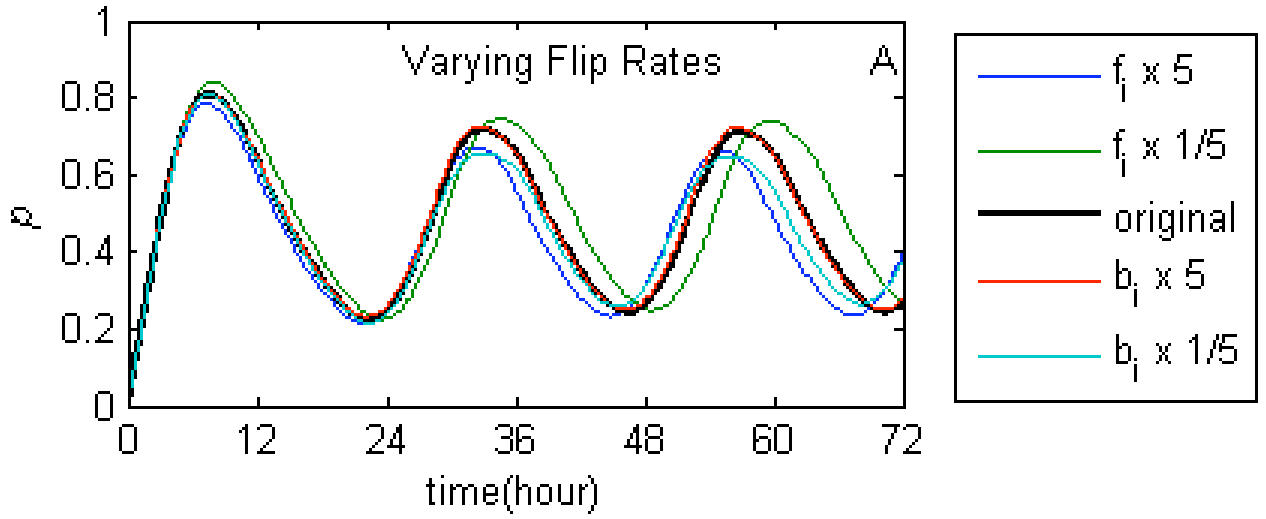
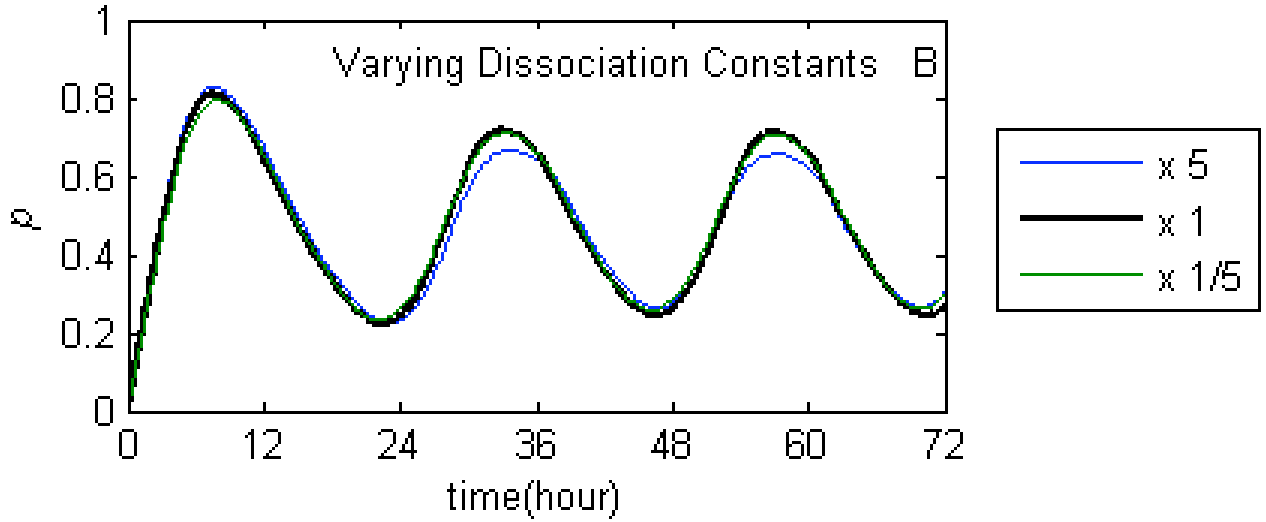
$$K_d << 1 \Rightarrow \frac{[A]}{K_d + [A]} \approx 1$$

T.C. Model


Varying Flip Rates
A
Q
time(hour)
0
0.2
0.4
0.6
0.8
1
0
12
24
36
48
60
72
f_i x 5
f_i x 1/5
original
b_i x 5
b_i x 1/5


Varying Dissociation Constants
B
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time(hour)
0
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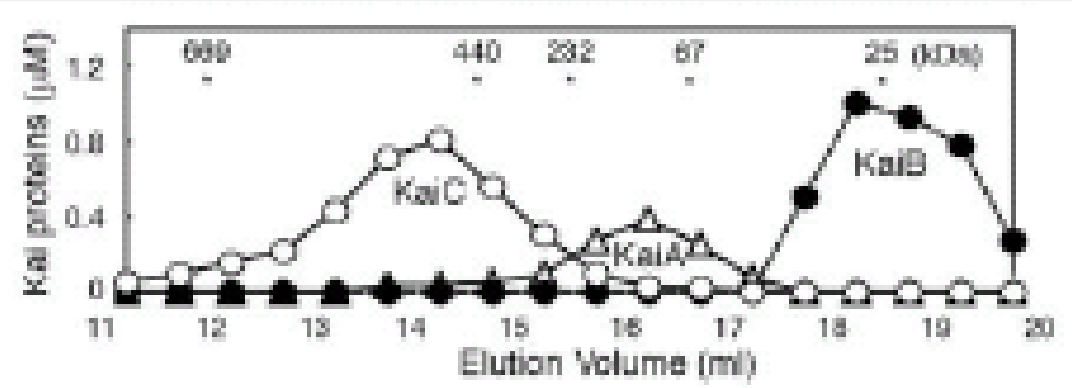
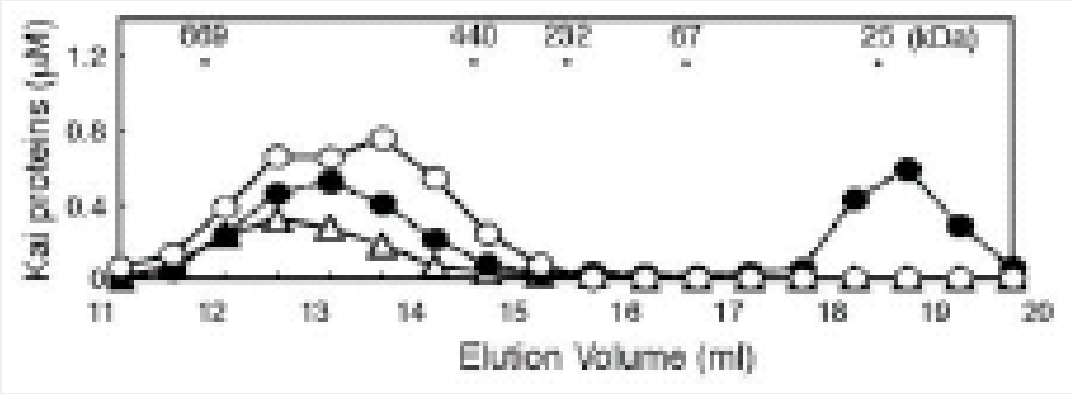
60
72
x 5
x 1
x 1/5

Temperature Compensation



t = 0 (reference)



t = 36 hrs.

geyama *et al.*,
l. Cell 2006

Temperature Compensation

- Mechanism makes predictions
  - No free KaiA
  - Period unchanged when increase all concentrations.

Kageyama *et al.*, *Mol. Cell* 2006

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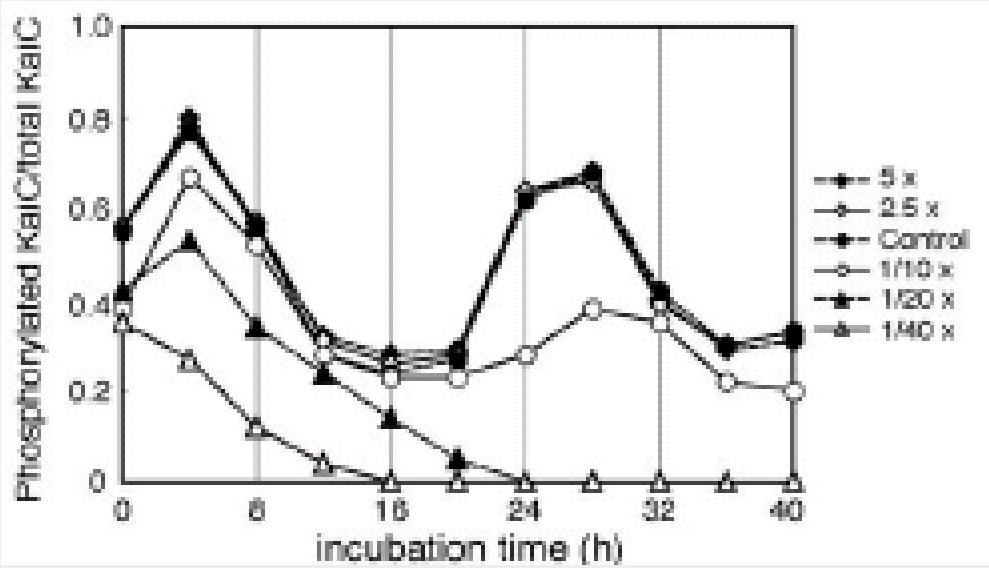


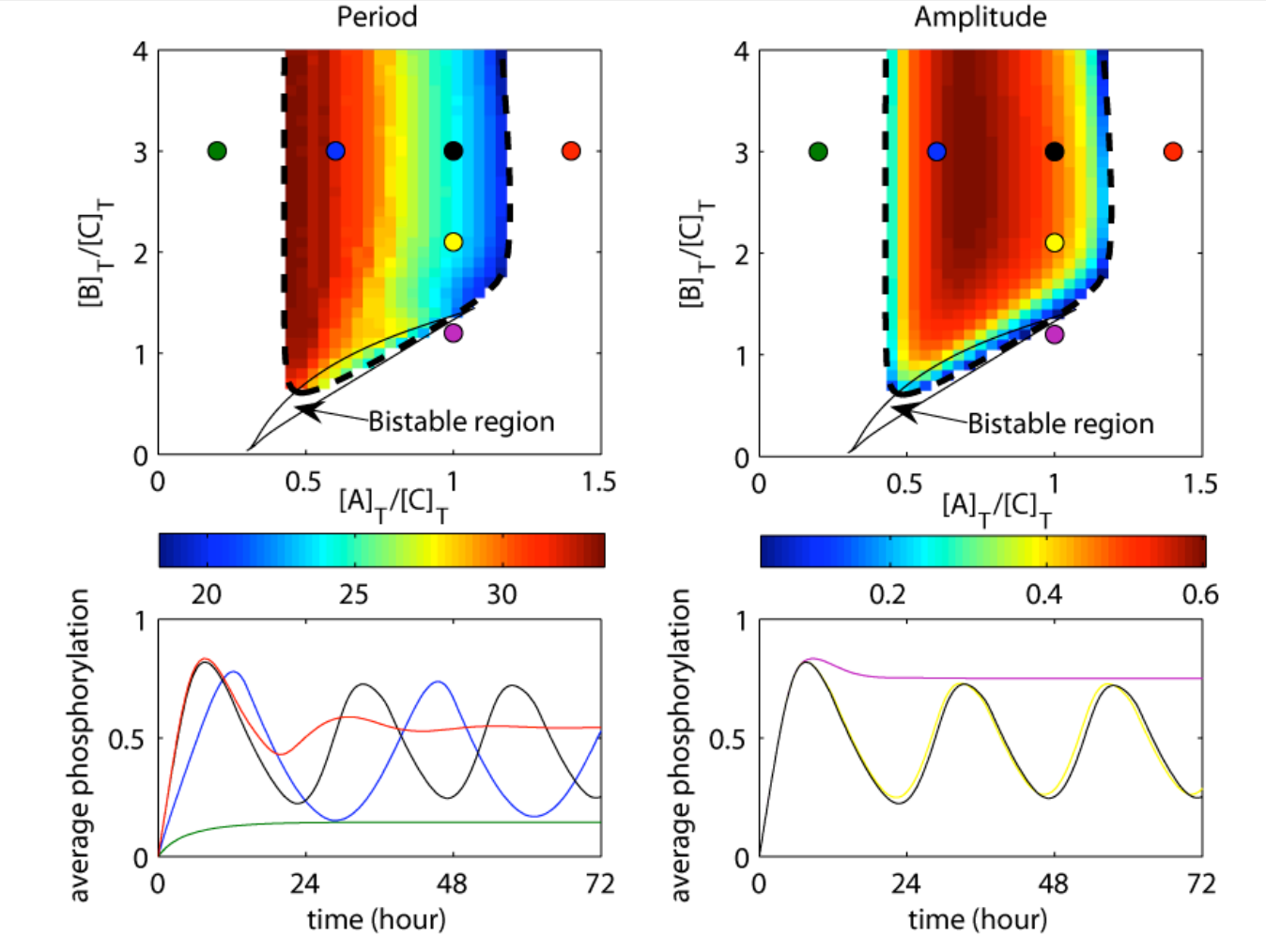
Kageyama *et al.*, *Mol. Cell* 2006

Predictions

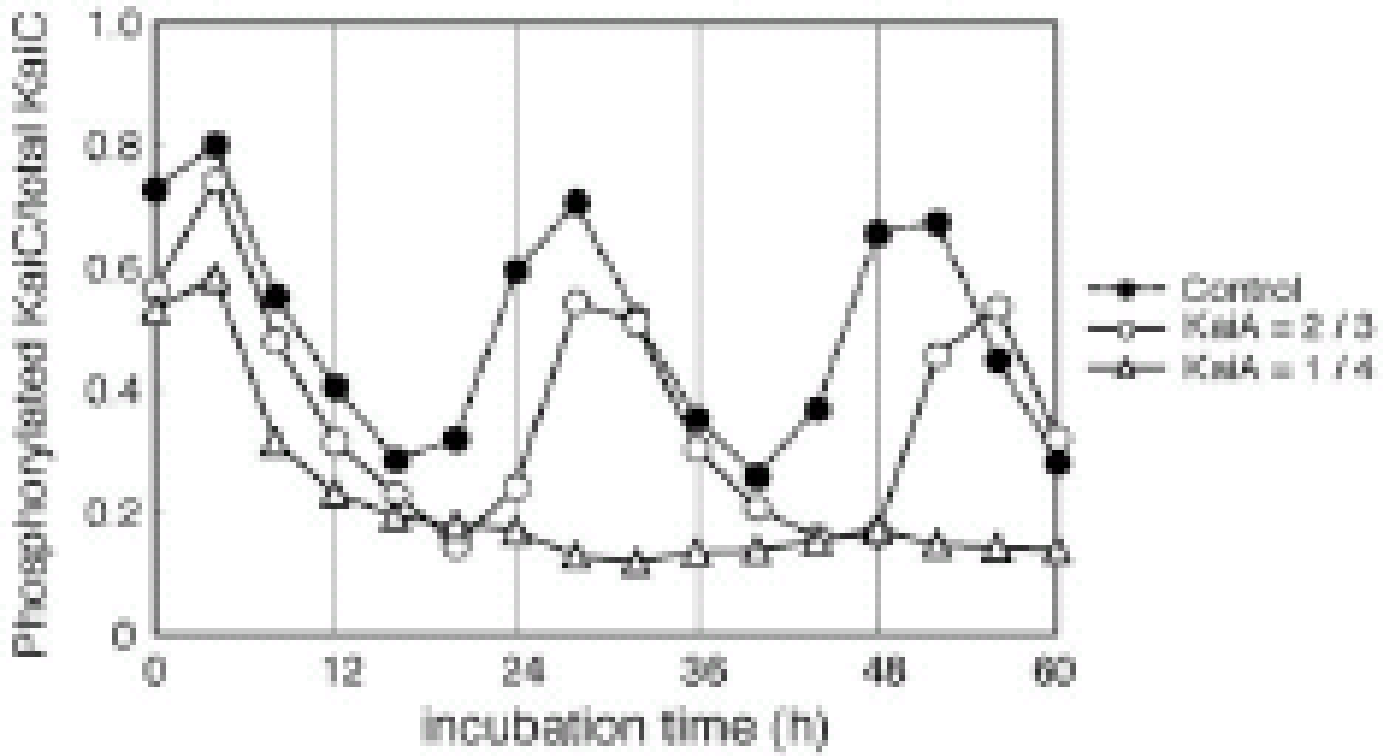
- KaiC has 2 conformations
  - Transitions between coupled to ATP hydrolysis/ phosphorylation.
  - This limits choice of rates, irreversibility,…
- Binding reactions driven to completion.

Predictions

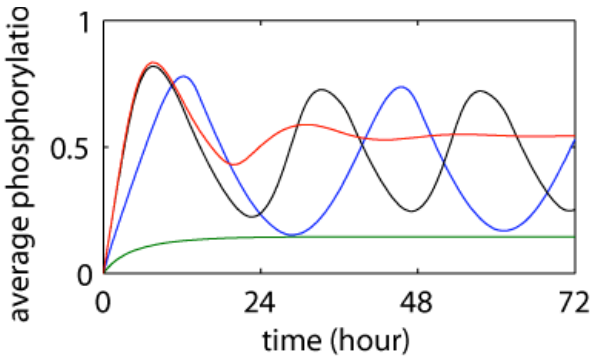
- Increasing [KaiB] leaves oscillations unaffected.
- Increasing [KaiA] destroys oscillations.

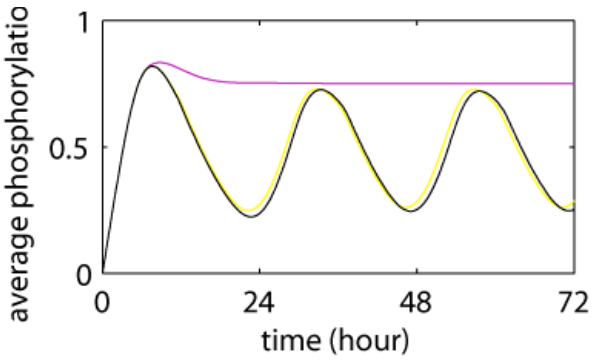


Predictions



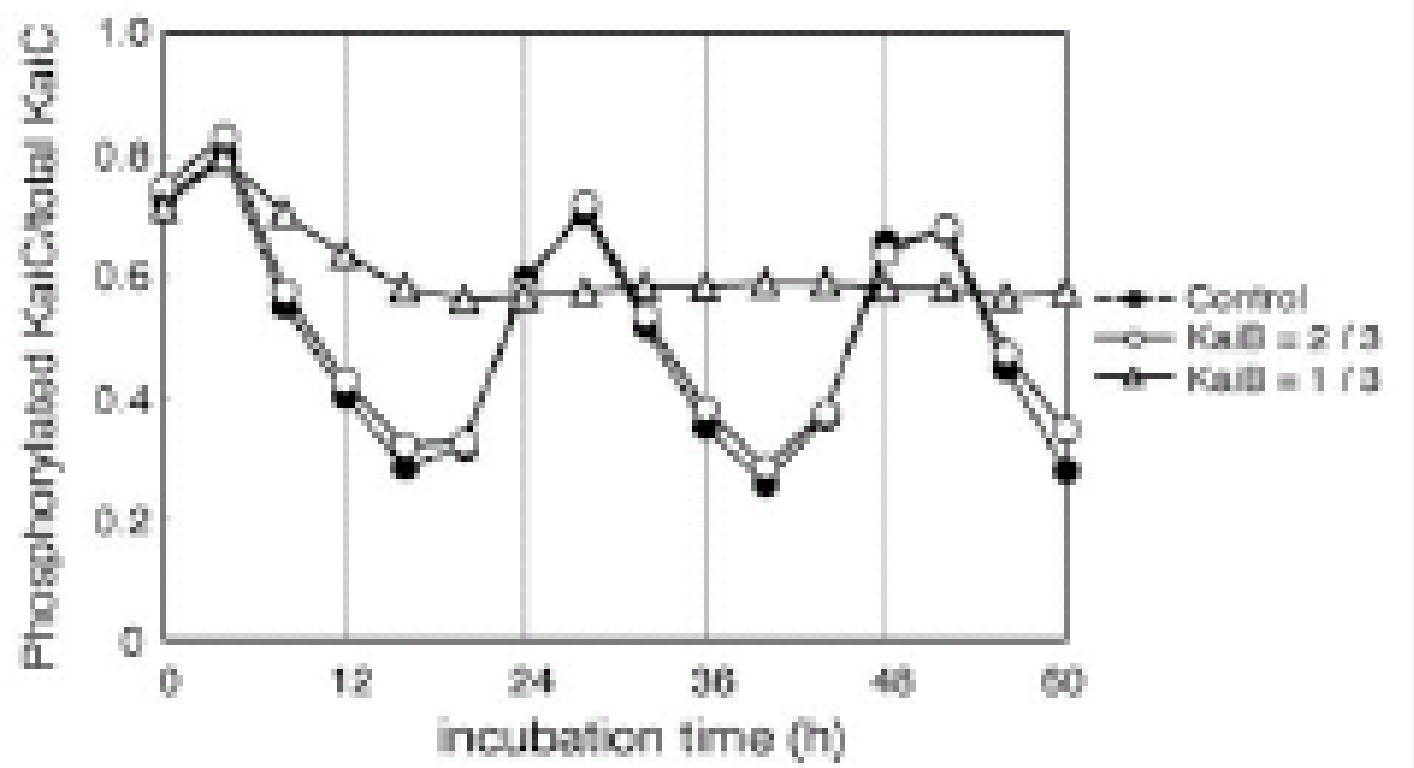
Kageyama *et al.*, *Mol. Cell* 2006

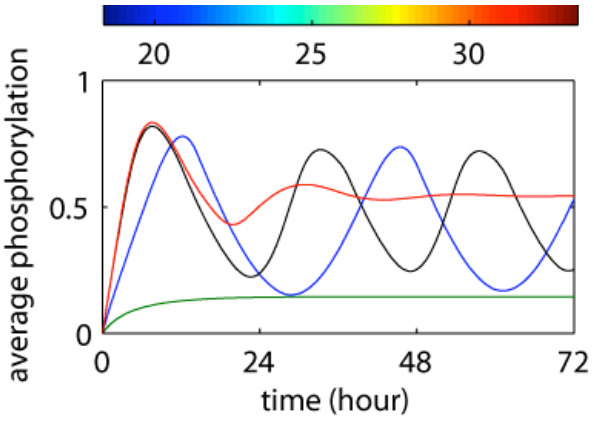


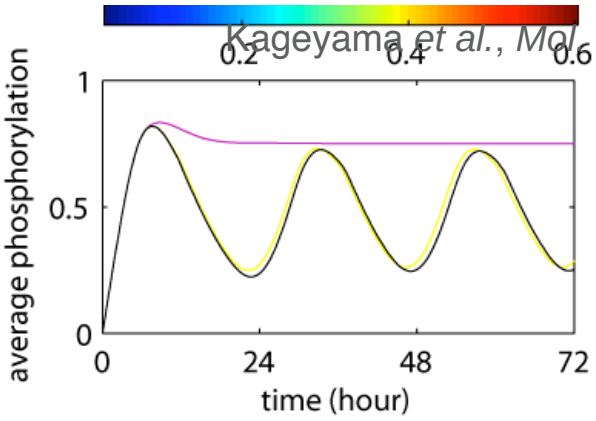


Predictions

- Inc d.
- Inc







Kageyama *et al.*, *Mol Cell* 2006

Summary

- Challenge: Mechanism for "minimal" protein oscillator.
  - Only 3 purified proteins.
  - Only 1 reaction cycle driven out of equilibrium.
- Proposal: Synchronization of molecular cycles via **differential affinity**.
- Predict: Increasing [KaiA], but not [KaiB], destroys oscillations.
- Outlook.
  - *In vivo*? Lower [KaiA], transcriptional feedback.
  - Evolution: No KaiA in ~50% of cyanobacteria!