Mathematical basis of stability analysis

$$\dot{x} = f(x, y)$$
$$\dot{y} = g(x, y)$$

system of two coupled differential equations

step 1 ↓

find nullclines and fixed point(s)

$$\dot{x} = 0 \rightarrow f(x_o, y_o) = 0$$
$$\dot{y} = 0 \rightarrow g(x_o, y_o) = 0$$

step 2 ↓

consider small deviation from fixed point

$$\tilde{x} \equiv x - x_o$$
$$\tilde{y} \equiv y - y_o$$

$$\tilde{x} \equiv x - x_o$$
$$\tilde{y} \equiv y - y_o$$

consider small deviation from fixed point

step 3 ↓

linearize around fixed point(s)

$$\dot{x} \approx \tilde{x} \left.\frac{\partial f}{\partial x}\right|_{(x_o, y_o)} + \tilde{y} \left.\frac{\partial f}{\partial y}\right|_{(x_o, y_o)} \equiv a\tilde{x} + b\tilde{y}$$

$$\dot{y} \approx \tilde{x} \left.\frac{\partial g}{\partial x}\right|_{(x_o, y_o)} + \tilde{y} \left.\frac{\partial g}{\partial y}\right|_{(x_o, y_o)} \equiv c\tilde{x} + d\tilde{y}$$

step 4 ↓

determine matrix A

$$A = \begin{bmatrix} a & b \\ c & d \end{bmatrix}$$

$$A = \begin{bmatrix} a & b \\ c & d \end{bmatrix}$$

determine matrix A

step 5 ↓

determine trace and determinant of A:

$$\tau = trace(A) = a + d$$

$$\Delta = \det(A) = ad - bc$$

step 6 ↓

determine stability of fixed point

only if $\tau < 0$ and $\Delta > 0$, (x_o, y_o) is a stable fixed point

!!! be careful: only valid for 2 dimensional systems !!!

Last lectures: **Genetic Switches**

L3-4: Naturally occurring: lysis-lysogeny decision

L5-6: Engineered: genetic toggle switch

Switches are necessary for making ‘decisions’:

- development & differentiation (e.g. stem cells)
  what to be ?
- metabolism
  what to eat ?
- molecule synthesis (e.g amino acids)
  what to produce ?

time scales for genetic regulation ~ 10 min - hours

Images removed to due copyright considerations.

What if faster response is needed ?

- finding food
- chasing bait
- signal transduction

Image removed due to copyright considerations.

genetics is too slow !

Protein switches (active/inactive states)
(total amount active + inactive is constant, ignore gene expression)

timescales 1 ms - minutes

Introducing the H atom for signal transduction:

chemotaxis of *Escherichia coli*

Image removed due to copyright considerations.
See Alberts, Bruce, et al. Chapter 13 in *Molecular biology of the cell*.
4th ed. New York: Garland Science, 2002.

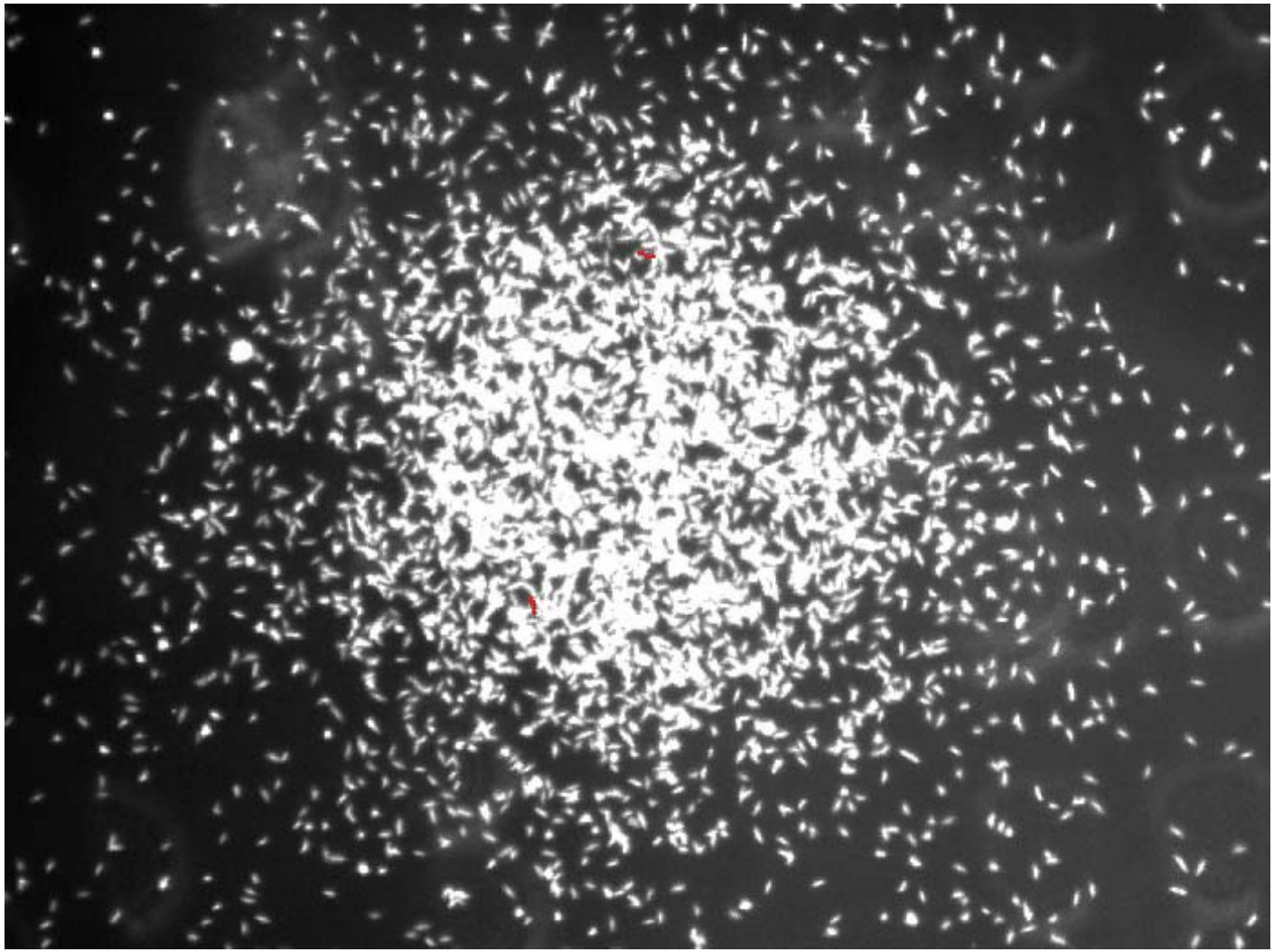


Figure 1A in Mittal, N., E. O. Budrene, M. P. Brenner, and A. Van Oudenaarden. "Motility of Escherichia coli cells in clusters formed by chemotactic aggregation." *Proc Natl Acad Sci U S A*. 100, no. 23 (Nov 11, 2003): 13259-63.

cell length ~ 1-2 μm, diameter ~ 0.5 μm

Images removed due to copyright considerations.

The Flagellum

Image removed due to copyright considerations.

Absence of chemical attractant

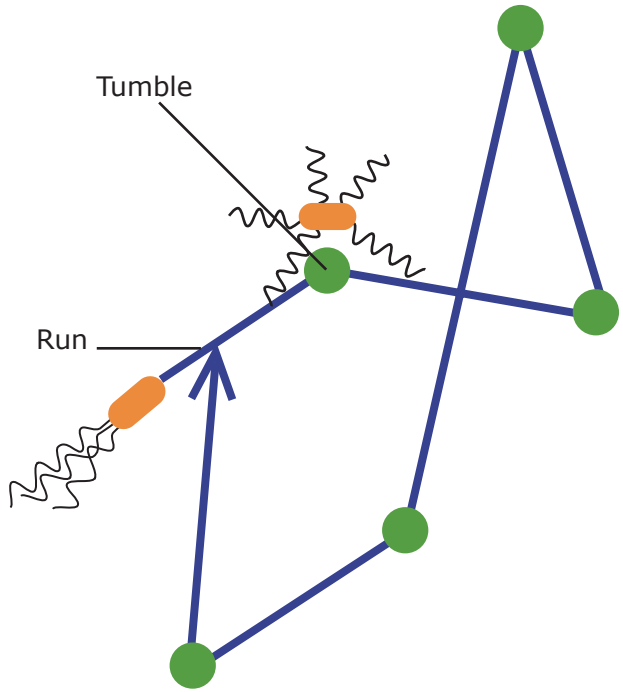
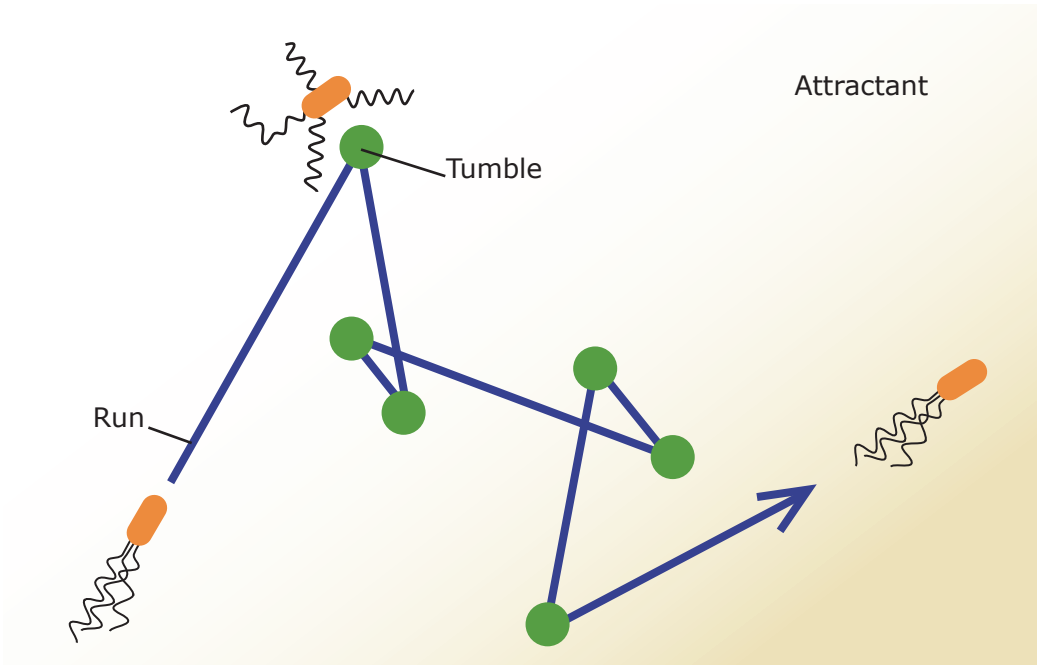


Image by MIT OCW.

Presence of chemical attractant



Chemical Gradient Sensed in a Temporal Manner

Image by MIT OCW.

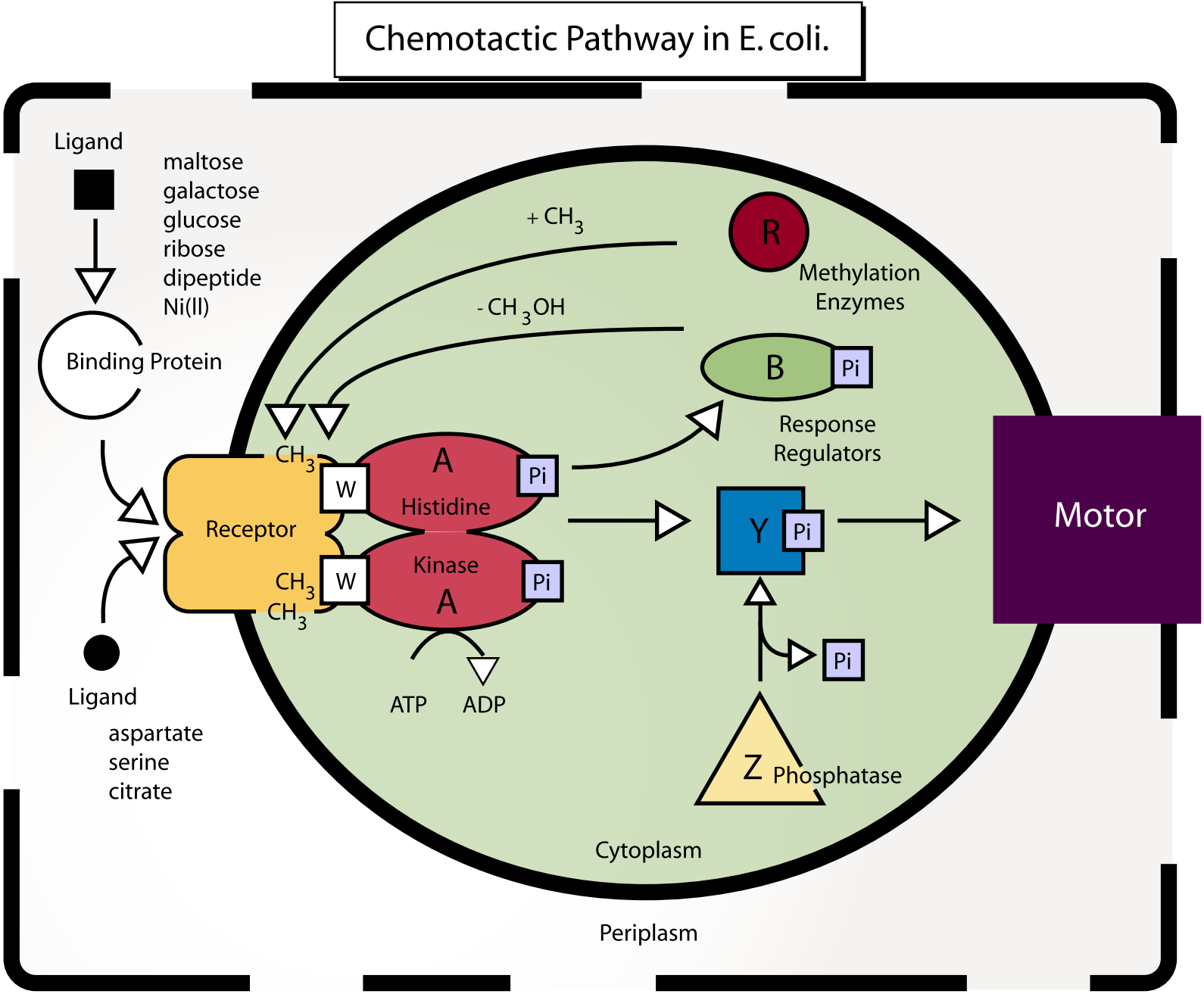


Image by MIT OCW. After figure 4 in Falke, J. J., R. B. Bass, S. L. Butler, S. A. Chervitz, and M. A. Danielson. "The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes." *Annu Rev Cell Dev Biol* 13 (1997): 457-512.

Chemotactic pathway in *E. coli*
Towards more complex system networks.

Image removed due to copyright considerations.

Proteins in the chemotactic network can be modified in differents ways:

I Phosphorylation (CheA, CheY, CheB)
II Methylation (Tar receptor)

Image removed due to copyright considerations.

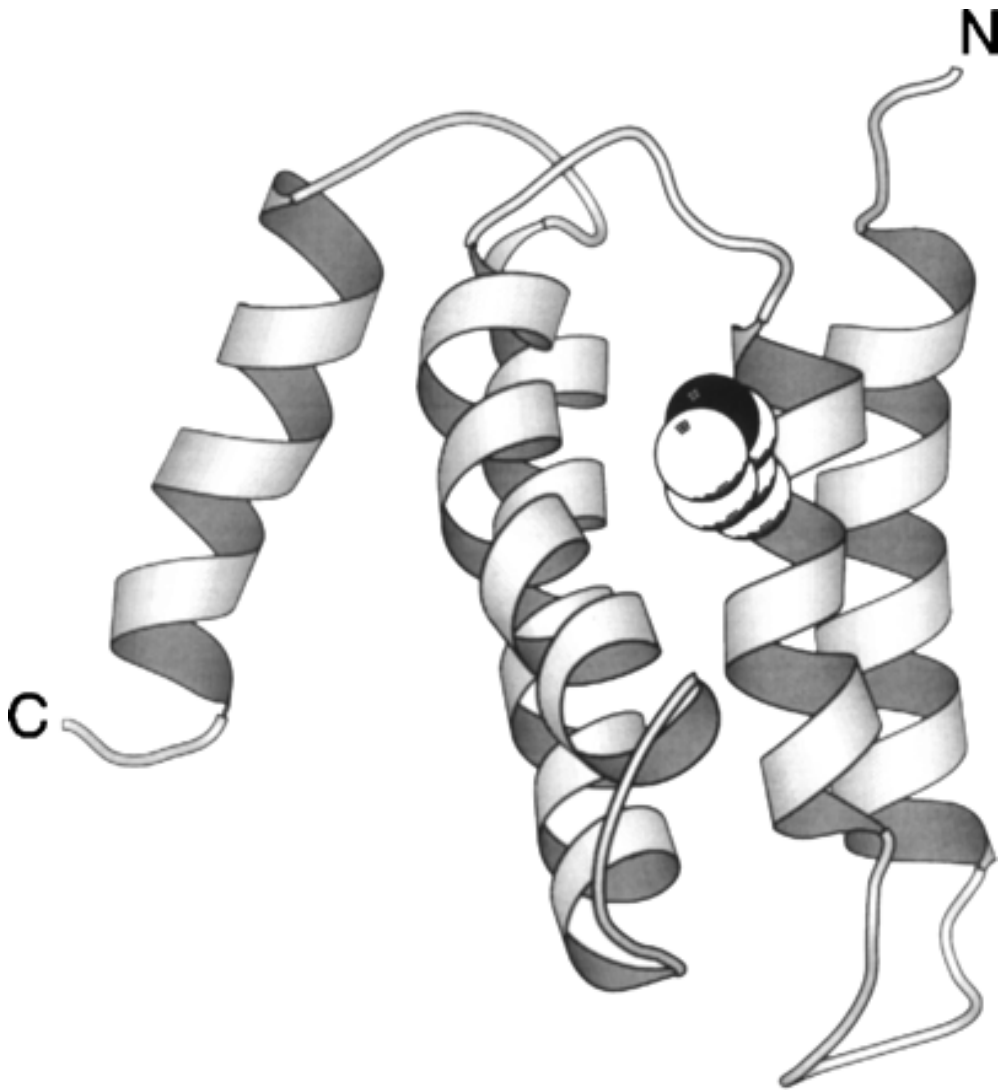
I Phosphorylation (CheA, CheY, CheB)

CheA (protein kinase), uses ATP to phosphorylate one of its histidines.

$$\mathrm{CheA} + \mathrm{ATP} \leftrightarrow \mathrm{CheA_p} + \mathrm{ADP}$$

CheA ($\mathbf{CheA_p}$)is bound to the Tar receptor through an adapter protein **CheW**. **CheW** is not known to have any enzymatic activity. (these proteins are sometimes called 'scaffolding protein')

$\mathbf{CheA_p}$ is unstable and transfers its phosphoryl group to CheY (highly soluble, diffuses through the cytoplasm



CheA
His48

Falke, J. J., R. B. Bass, S. L. Butler, S. A. Chervitz, and M. A. Danielson.
"The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes." *Annu Rev Cell Dev Biol* 13 (1997): 457-512.

I Phosphorylation (CheA, CheY, CheB)

autophosphorylation: $\mathrm{CheA} + \mathrm{ATP} \leftrightarrow \mathrm{CheA_p} + \mathrm{ADP}$

phosphoryltransfer: $\mathrm{CheA_p} + \mathrm{CheY} \leftrightarrow \mathrm{CheA} + \mathrm{CheY_p}$

CheYp binds to the motor (FliM),
motor rotates CW (= tumbles)

logic:

high levels of CheA -> high levels of CheYp

(lots of tumbles)

low levels of CheA -> low levels of CheYp

(straight swimming)

Image removed due to copyright considerations.

CheZ dephosphorylates $CheY_p$
(opposite function as CheA)

$$CheY_p + CheZ \leftrightarrow CheY + CheZ_p$$

logic:
high levels of CheZ -> low levels of CheYp

(straight swimming)

II Methylation (tar receptor)

Image removed due to copyright considerations.

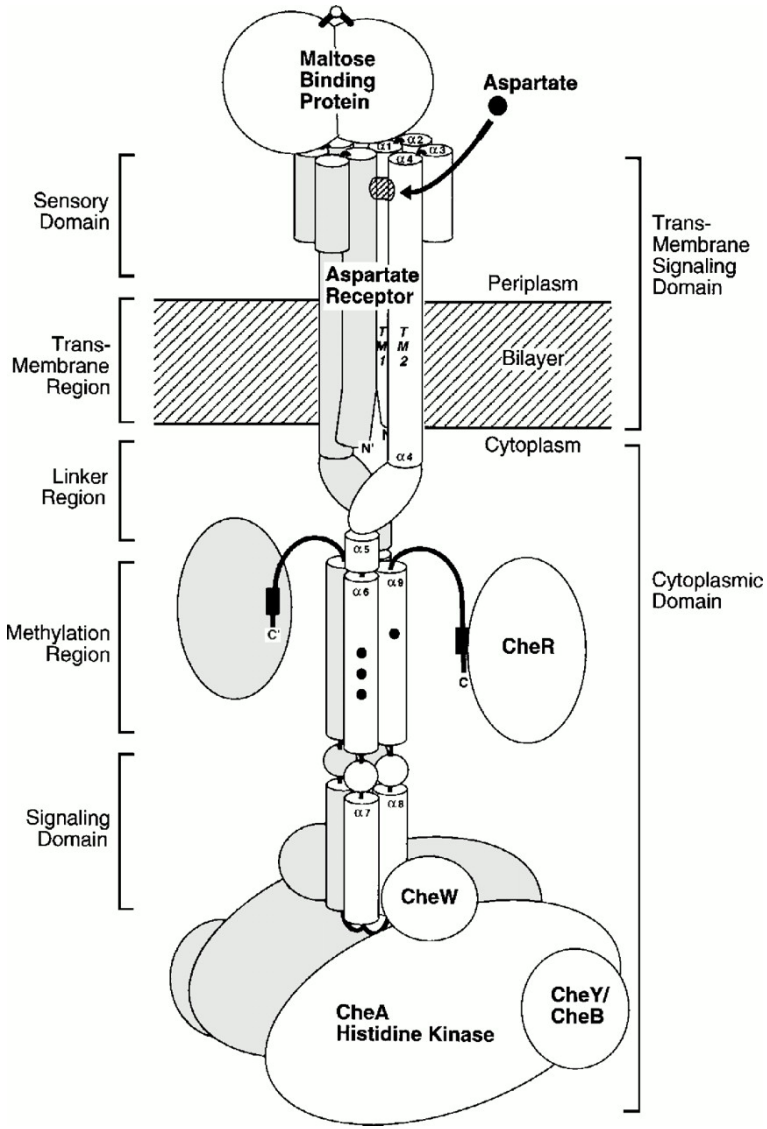
CheR adds methyl group
$CheB_p$ removes methyl group

phosphorylation state of CheB is controlled by CheA

Methylation - Phosphorylation coupling

Image removed due to copyright considerations.

phosphorylation state of CheB
is controlled by CheA



Falke, J. J., R. B. Bass, S. L. Butler, S. A. Chervitz, and M. A. Danielson.
"The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes." *Annu Rev Cell Dev Biol* 13 (1997): 457-512.

Role of ligand binding

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The rate of CheA phosphorylation is stimulated by unoccupied receptors

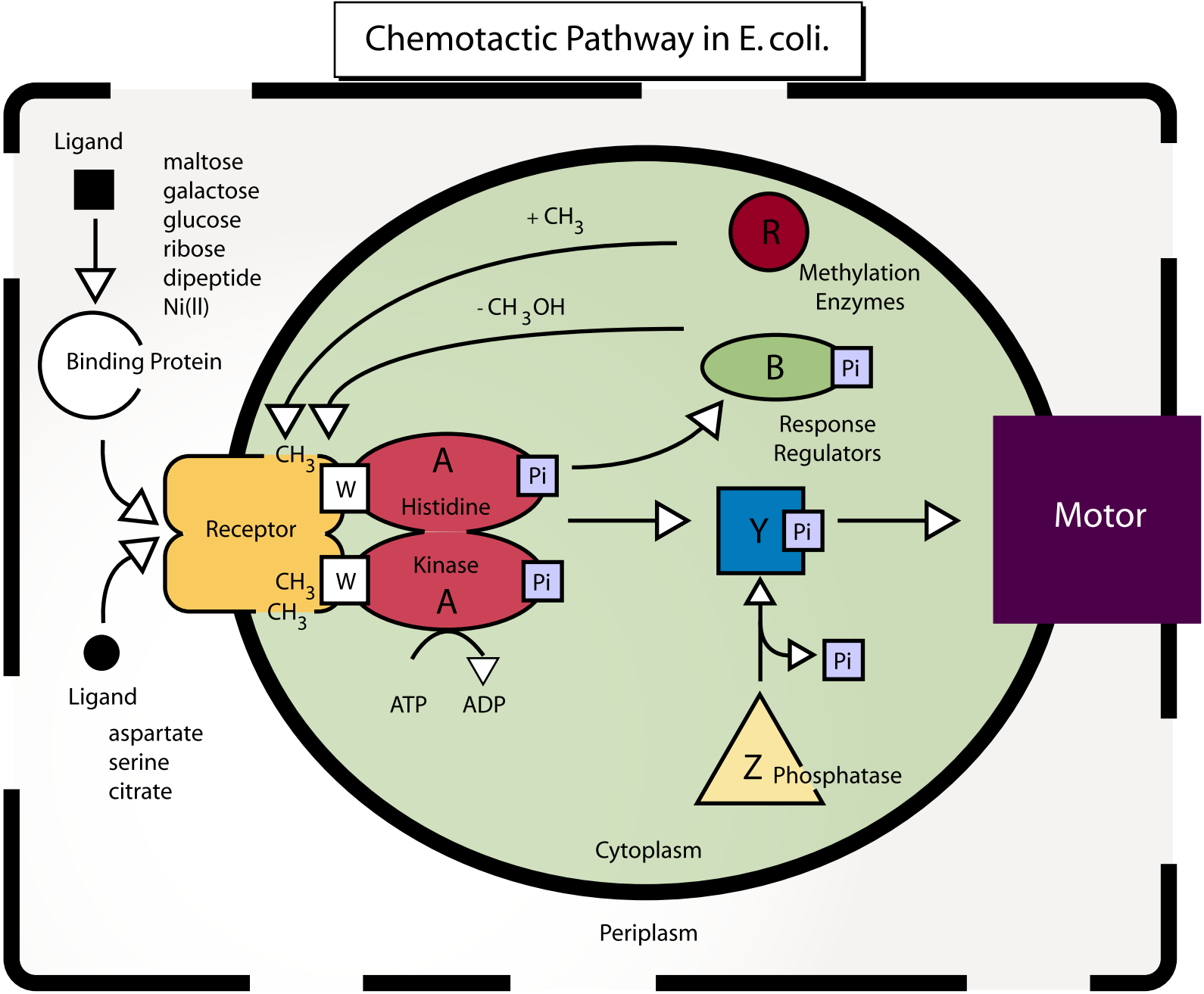
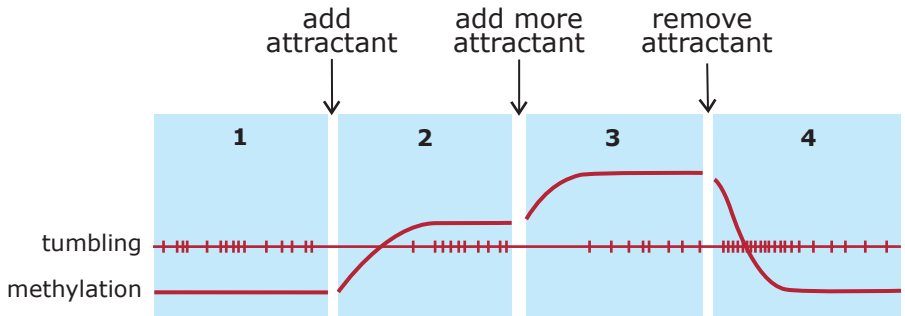


Image by MIT OCW. After figure 4 in Falke, J. J., R. B. Bass, S. L. Butler, S. A. Chervitz, and M. A. Danielson. "The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes." *Annu Rev Cell Dev Biol* 13 (1997): 457-512.

why is this all so complex ?



Correlation of Receptor Methylation with Behavioral Response

Image by MIT OCW.

methylation is important for adaptation
(~ background subtraction)

E. coli can sense aspartate from 10 nM - 1 mM
and sense changes as small as 0.1%

Before starting with the modeling,
first let's look at some recent experiments

Alon et al. Nature **397**,168 (1999)
Cluzel et al. Science **287**, 1652 (2000)
Sourjik et al., PNAS **99,** 123 (2002)
PNAS **99**, 12669 (2002)
Nature **428**, 439 (2004)

Remember scientists have been working on
E. coli chemotaxis for about 100 years now

Image removed due to copyright considerations. See figure 1 in Cluzel, P., M. Surette, and S. Liebler. "An ultrasensitive bacterial motor revealed by monitoring signaling proteins in single cells." *Science* 287, no. 5458 (Mar 3, 2000): 1652-5.

Single cell chemotactic analysis

correlation CW bias & CheY-P gene expression

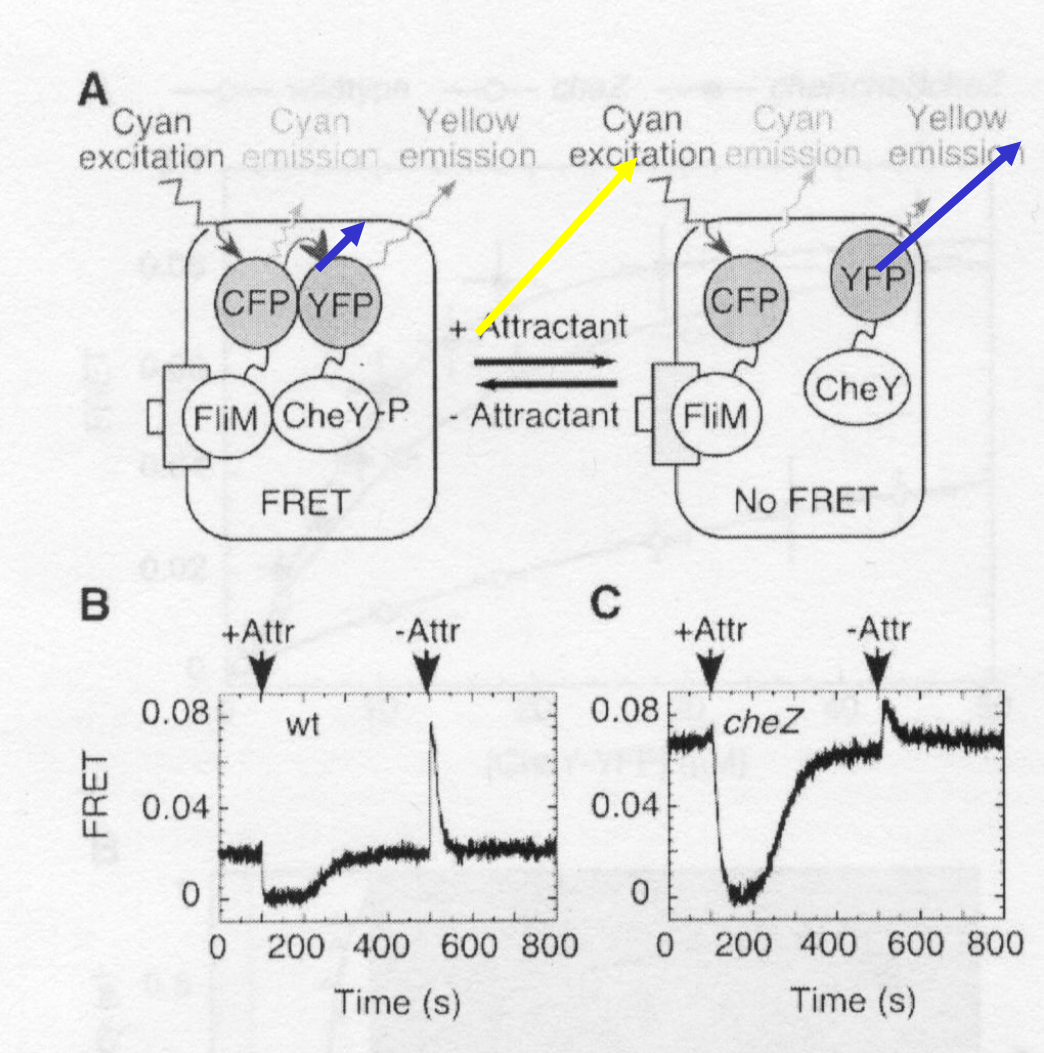
cells have plasmids with CheY-GFP under inducible promoter

assumption: all CheY is phosphorylated

strain: CheY-, CheZ-, CheB-

Hill #: ~10

Image removed due to copyright considerations. See figure 1 in Cluzel, P., M. Surette, and S. Liebler. "An ultrasensitive bacterial motor revealed by monitoring signaling proteins in single cells." *Science* 287, no. 5458 (Mar 3, 2000): 1652-5.



low YFP/CFP: unbound

high YFP/CFP: bound

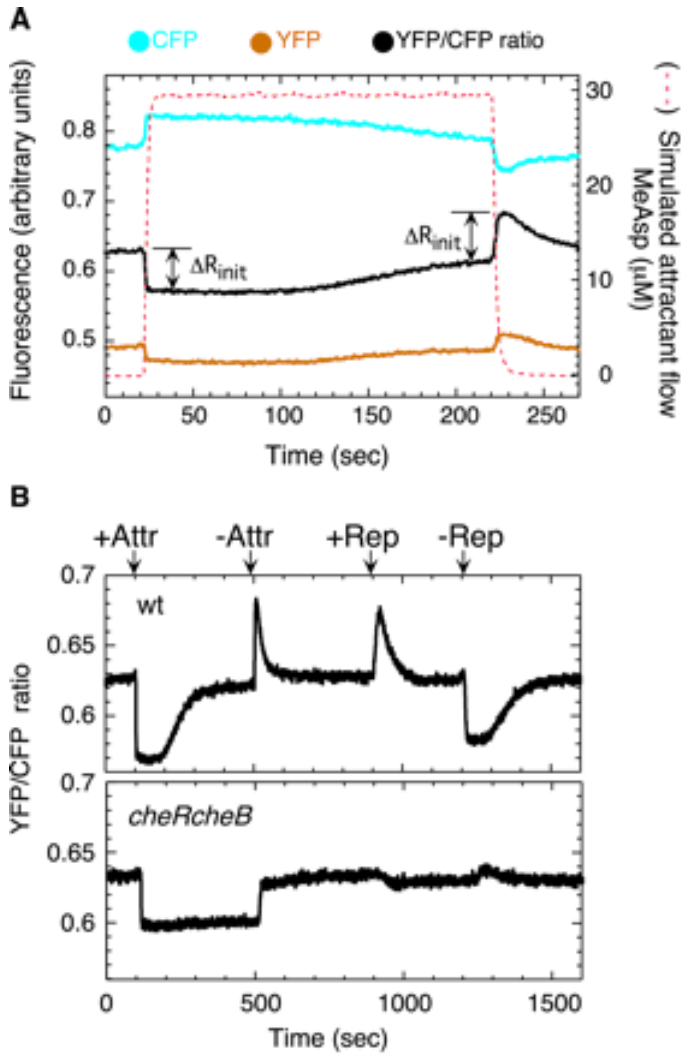
FRET (fluorescence resonant transfer)

Figures 1A, 1B in Sourjik, V., and H. C. Berg. "Binding of the Escherichia coli response regulator CheY to its target measured in vivo by fluorescence resonance energy transfer." *Proc Natl Acad Sci U S A* 99, no. 20 (Oct 1, 2002): 12669-74.

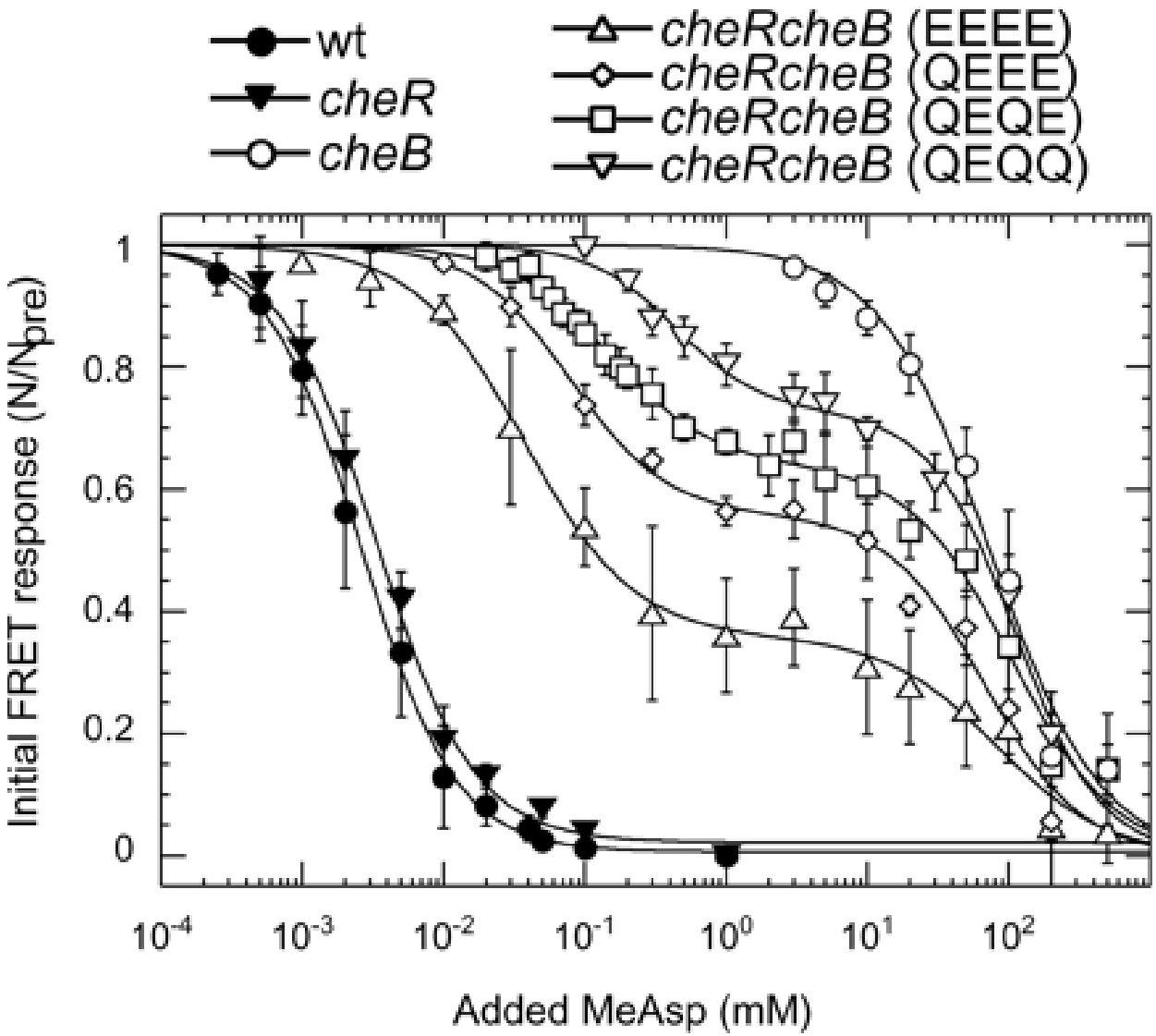
CheY-YFP (yellow)
CheZ-CFP (blue

CheZ binds only to CheYp !!

adding attractant leads to immediate lower concentration of $CheY_p$-CheZ complex, lower [CheYp], less tumbling



Figures 1A and 1B in Sourjik, V., and Berg HC. "Receptor sensitivity in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 99, no. 1 (Jan 8, 2002): 123-7.



Hill # ~ 1

Figure 2 in Sourjik, V., and Berg HC. "Receptor sensitivity in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 99, no. 1 (Jan 8, 2002): 123-7.

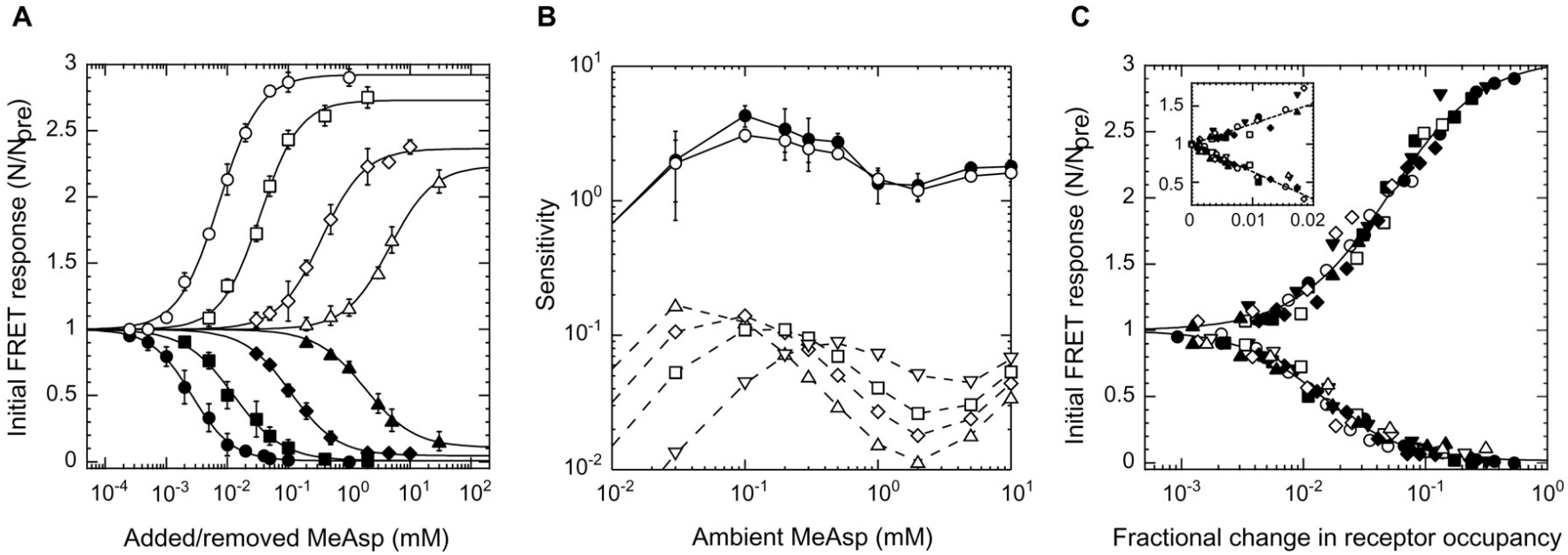
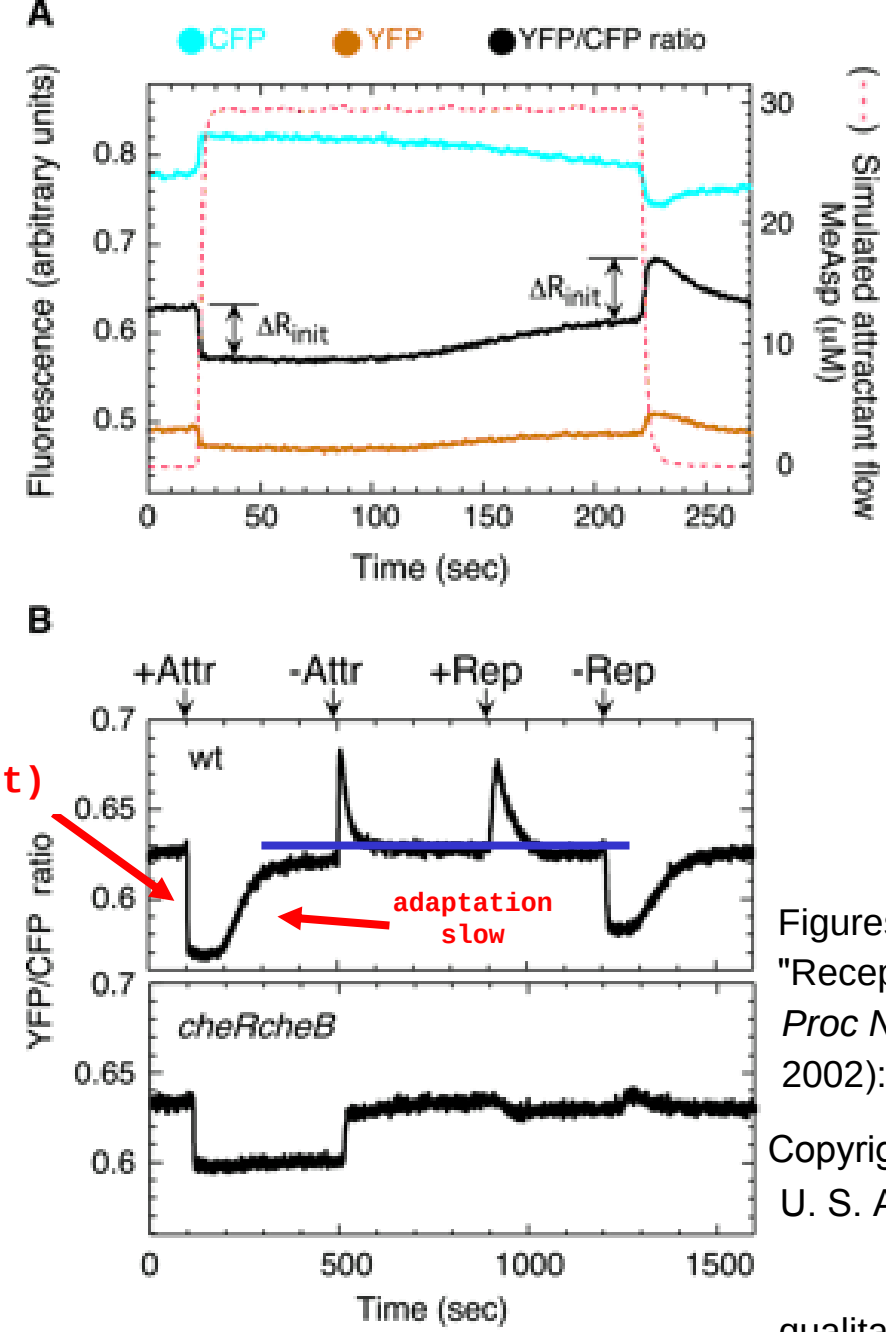


Figure 2 in Sourjik, V., and Berg HC. "Receptor sensitivity in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 99, no. 1 (Jan 8, 2002): 123-7.

amplification between receptors and CheYp: ~35

amplification between CheYp and motor: ~10

total amplification ~ 350
our models should reproduce this (hint: receptor clustering)



excitation(fast)

perfect adaptation

Figures 1a and 1 b in Sourjik, V., and Berg HC. "Receptor sensitivity in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 99, no. 1 (Jan 8, 2002): 123-7.

Models should also reproduce qualitative properties such as perfect adaptation

Images removed due to copyright considerations. See Figure 1 in Alon, U., M. G. Surette, N. Barkai, and S. Leibler. "Robustness in bacterial chemotaxis." *Nature* 397, no. 6715 (Jan 14, 1999): 168-71.

Perfect adaptation is robust against changes in Che-protein concentrations

Images removed due to copyright considerations. See Figure 2 in Alon, U., M. G. Surette, N. Barkai, and S. Leibler. "Robustness in bacterial chemotaxis." *Nature* 397, no. 6715 (Jan 14, 1999): 168-71.

not all parameters are robust !

Goal of next lecture is develop models that qualitatively and quantitative reproduce these phenomena, such as:

huge gain
sensitivity
perfect adaptation

All these effects are ubiquitous in signal transduction pathways in general.

‘Fine tuned model for perfect adaptation’

Spiro et al. PNAS **94**, 7263-7268 (1997)
A model of excitation and adaptation in bacterial chemotaxis

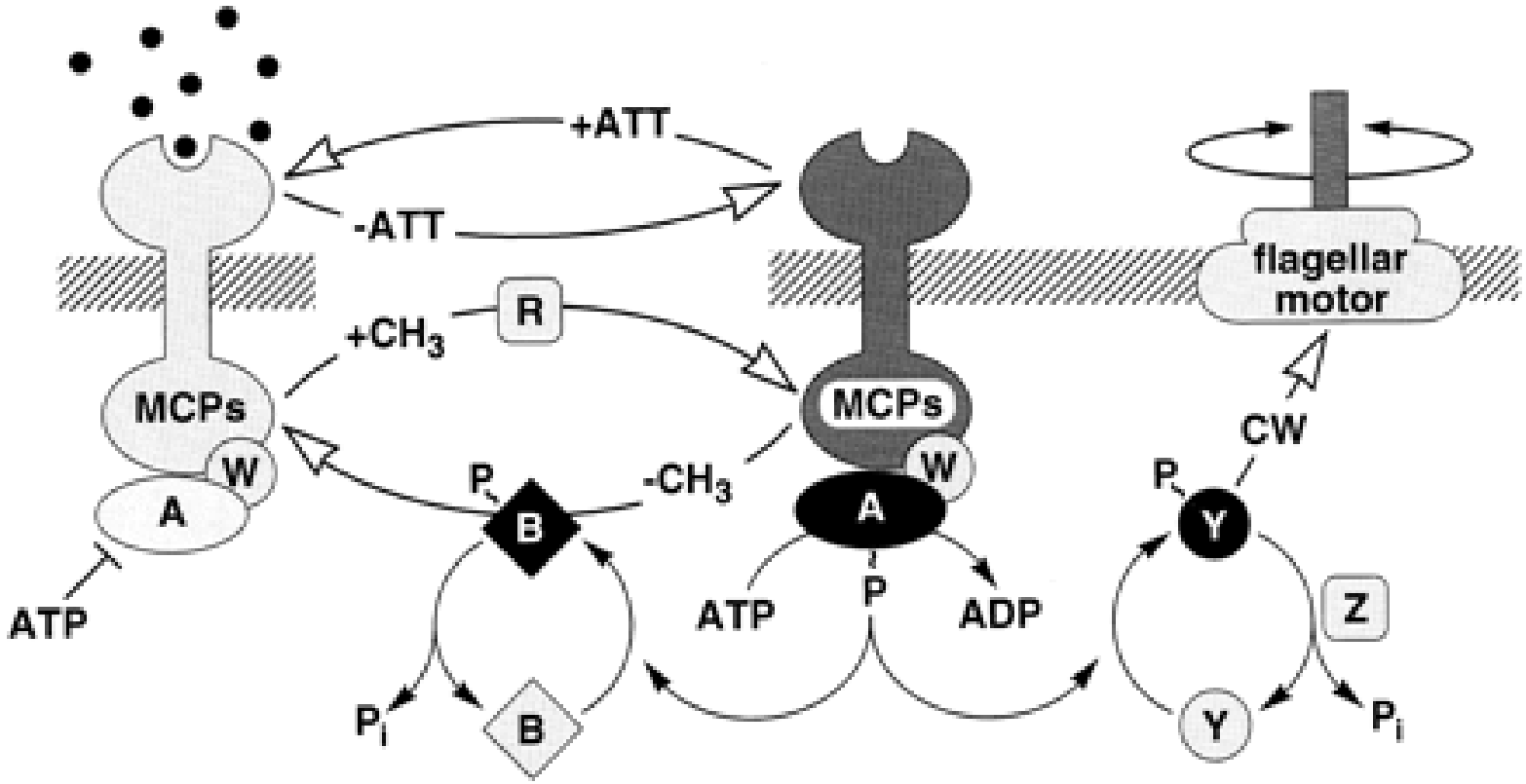


Figure 1 of Spiro P. A., J. S. Parkinson, and H. G. Othmer. "A model of excitation and adaptation in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 94, no. 14 (Jul 8, 1997): 7263-8.

key player: Tar-CheA-CheW complex

assumptions:

1. Tar is only receptor type, CheW and CheA always bound to Tar
2. Methylation occurs in specific order
3. Consider only 3 highest methylation states
4. Only $CheB_p$ demethylates
5. Phoshorylation of CheA does not affect ligand (un)binding
6. Tar-CheR binding does not affect ligand un(binding) and phosphorylation of CheA
7. CheZ is not regulated
8. Phosphotransfer from complex to CheY or CheB is not affected by occupancy or methylation state.

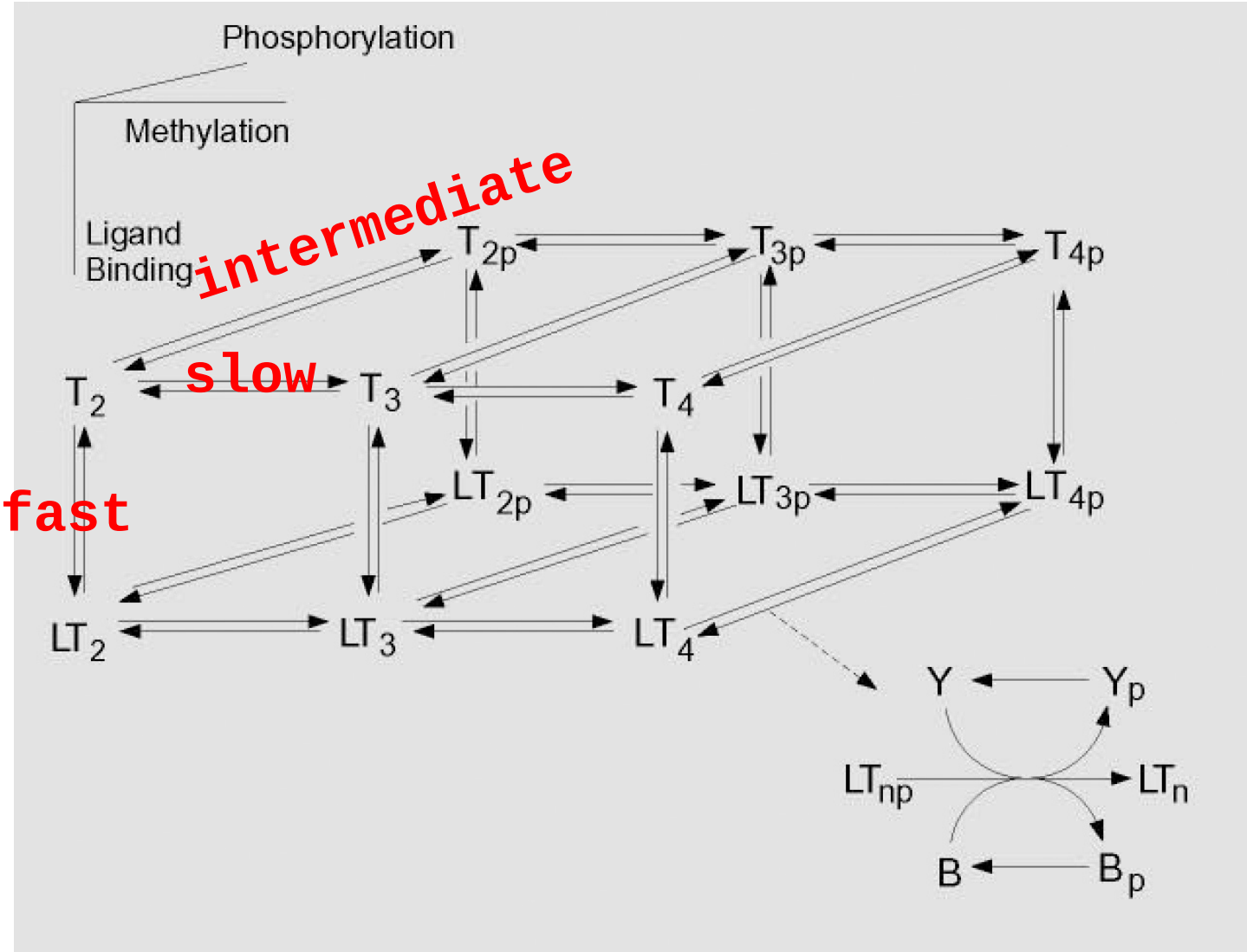


Figure 2 of Spiro P. A., J. S. Parkinson, and H. G. Othmer. "A model of excitation and adaptation in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 94, no. 14 (Jul 8, 1997): 7263-8.

Ligand bound states generally have lower autophosphoryalation rates

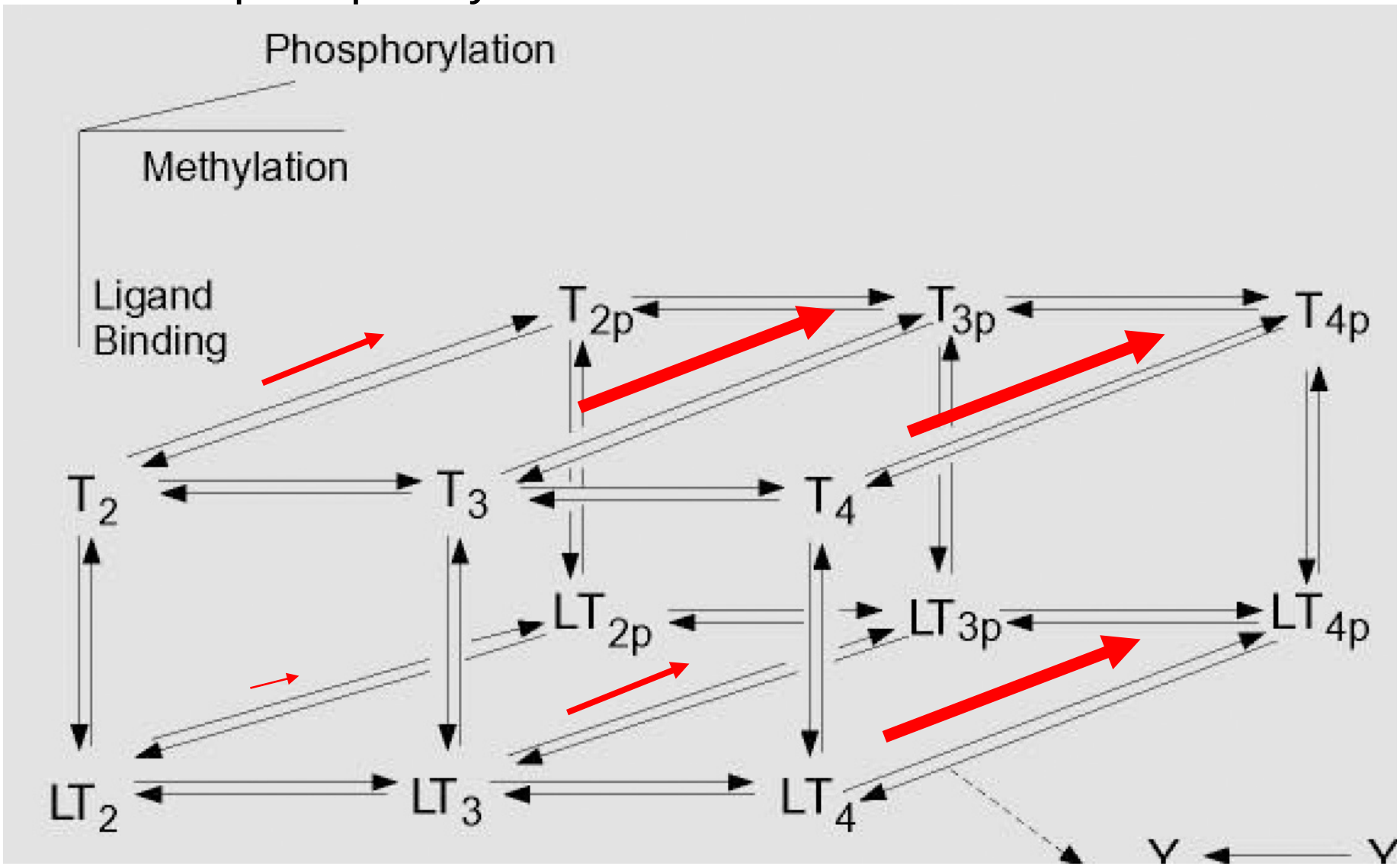


Figure 2 of Spiro P. A., J. S. Parkinson, and H. G. Othmer. "A model of excitation and adaptation in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 94, no. 14 (Jul 8, 1997): 7263-8.

CheR methylates ligand-bound states more rapidly

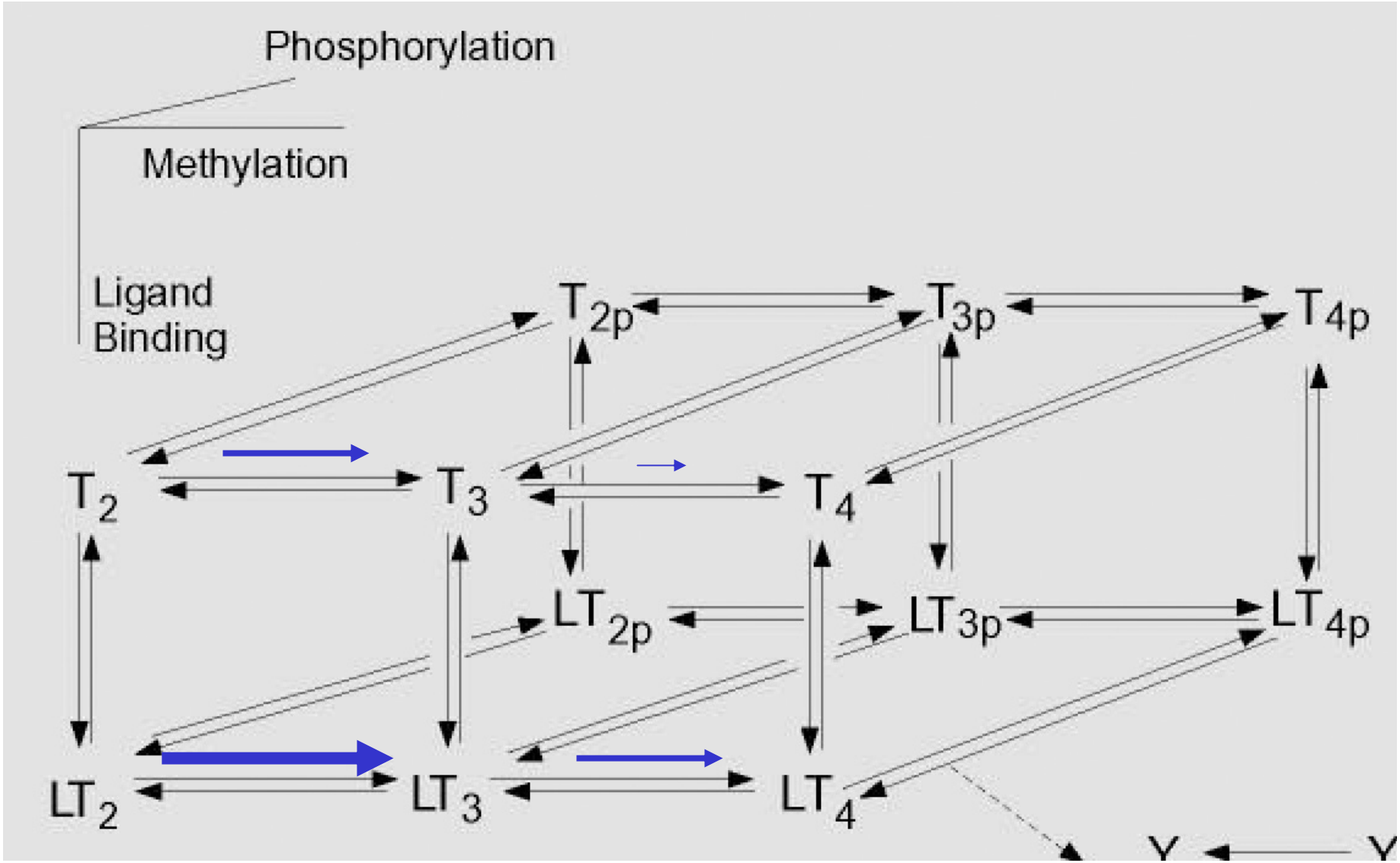


Figure 2 of Spiro P. A., J. S. Parkinson, and H. G. Othmer. "A model of excitation and adaptation in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 94, no. 14 (Jul 8, 1997): 7263-8.

Consider step in aspartate concentration
time ~ 1 ms, increase in ligand bound complex

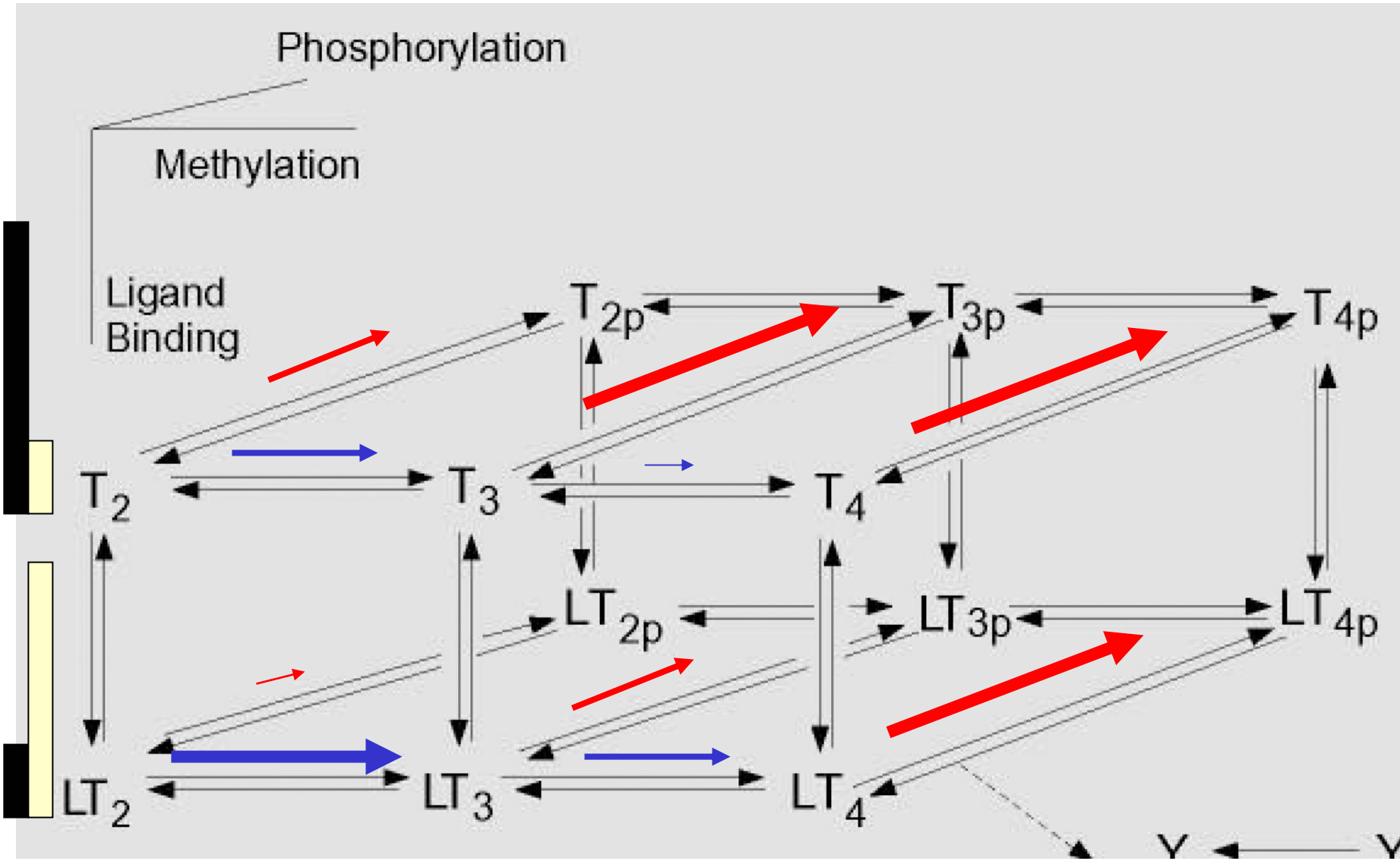


Figure 2 of Spiro P. A., J. S. Parkinson, and H. G. Othmer. "A model of excitation and adaptation in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 94, no. 14 (Jul 8, 1997): 7263-8.

time ~ 5 s, total # of phosphorylated complexes decreases gradually because ligand bound complexes do not autophoshorylate very well

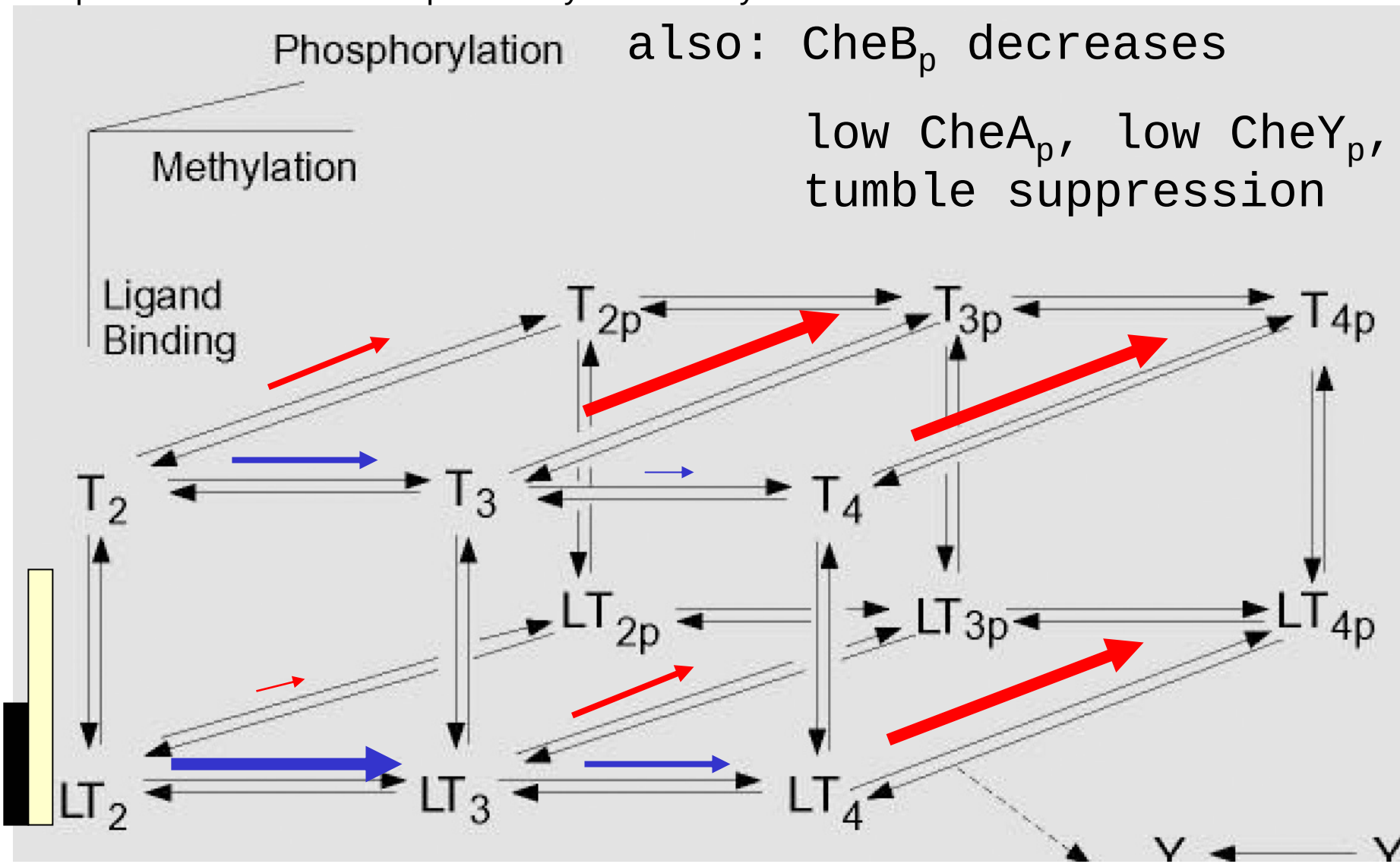


Figure 2 of Spiro P. A., J. S. Parkinson, and H. G. Othmer. "A model of excitation and adaptation in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 94, no. 14 (Jul 8, 1997): 7263-8.

time ~ 50 s, slowly the unbound complex methylate. Note that demethylation is switched because of low levels of $CheA_p$ (low $CheB_p$).

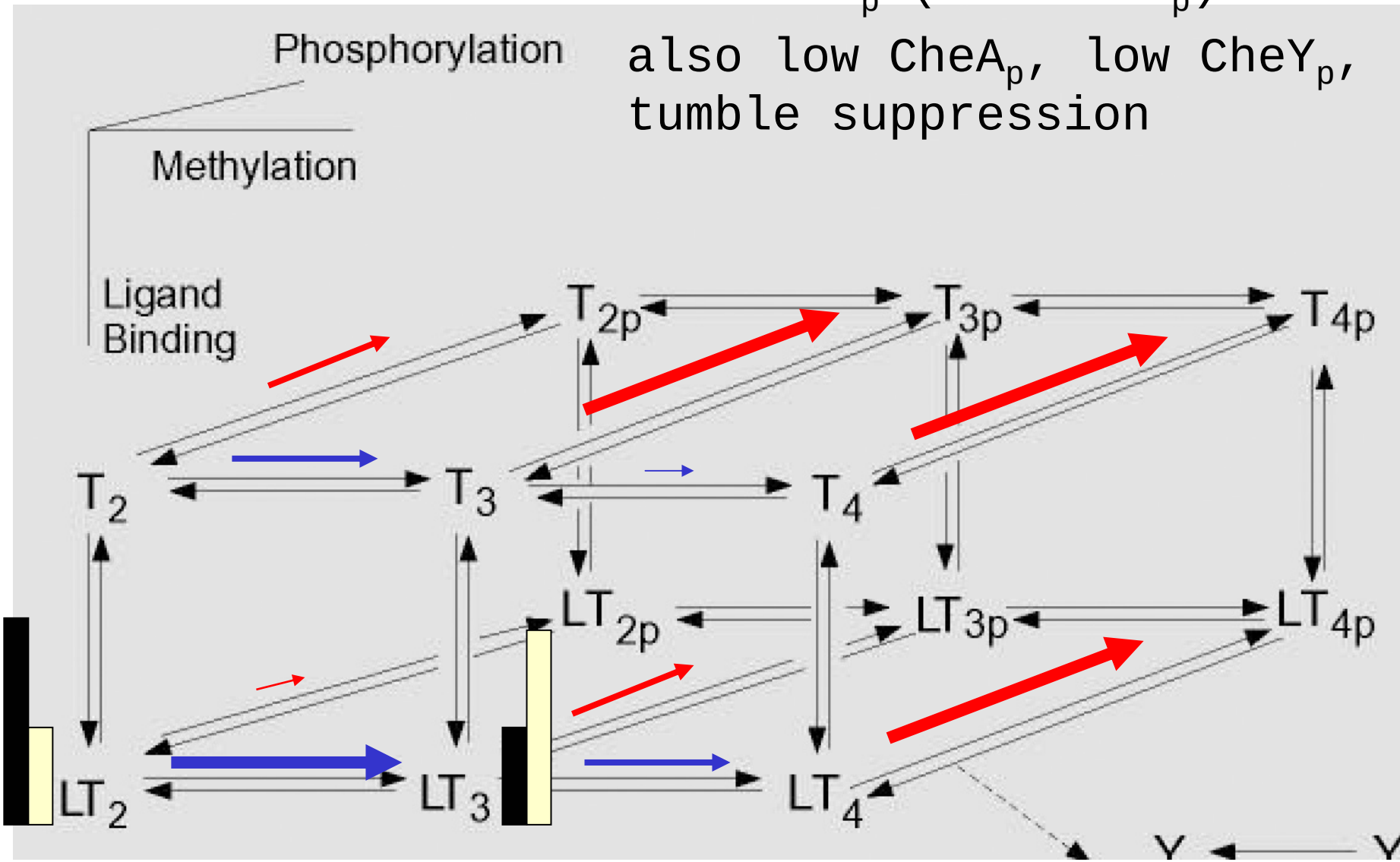


Figure 4 of Spiro P. A., J. S. Parkinson, and H. G. Othmer. "A model of excitation and adaptation in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 94, no. 14 (Jul 8, 1997): 7263-8.

Higher methylation states autophosphorylate easier, so slowly $CheA_p$ adapts to its initial level

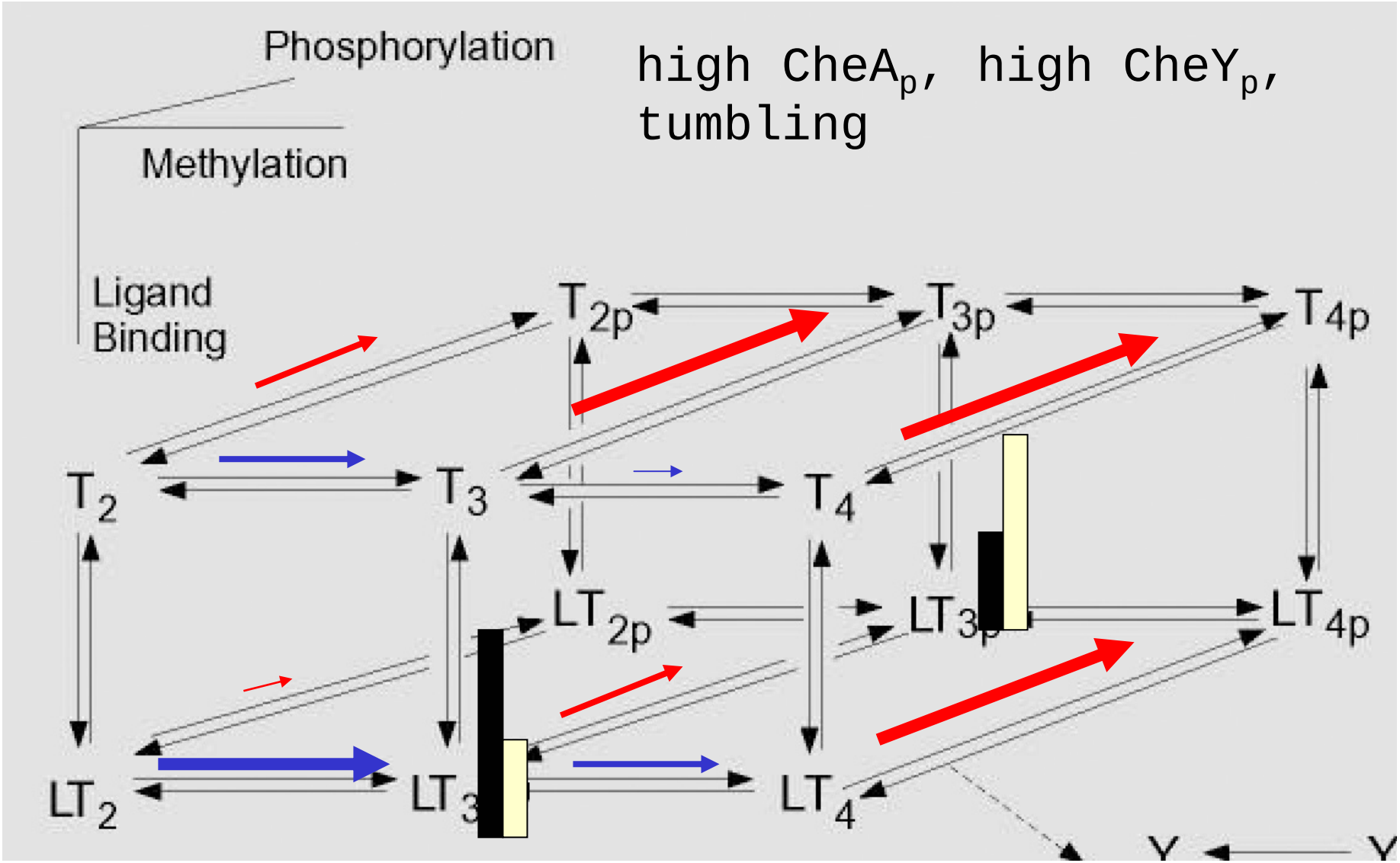
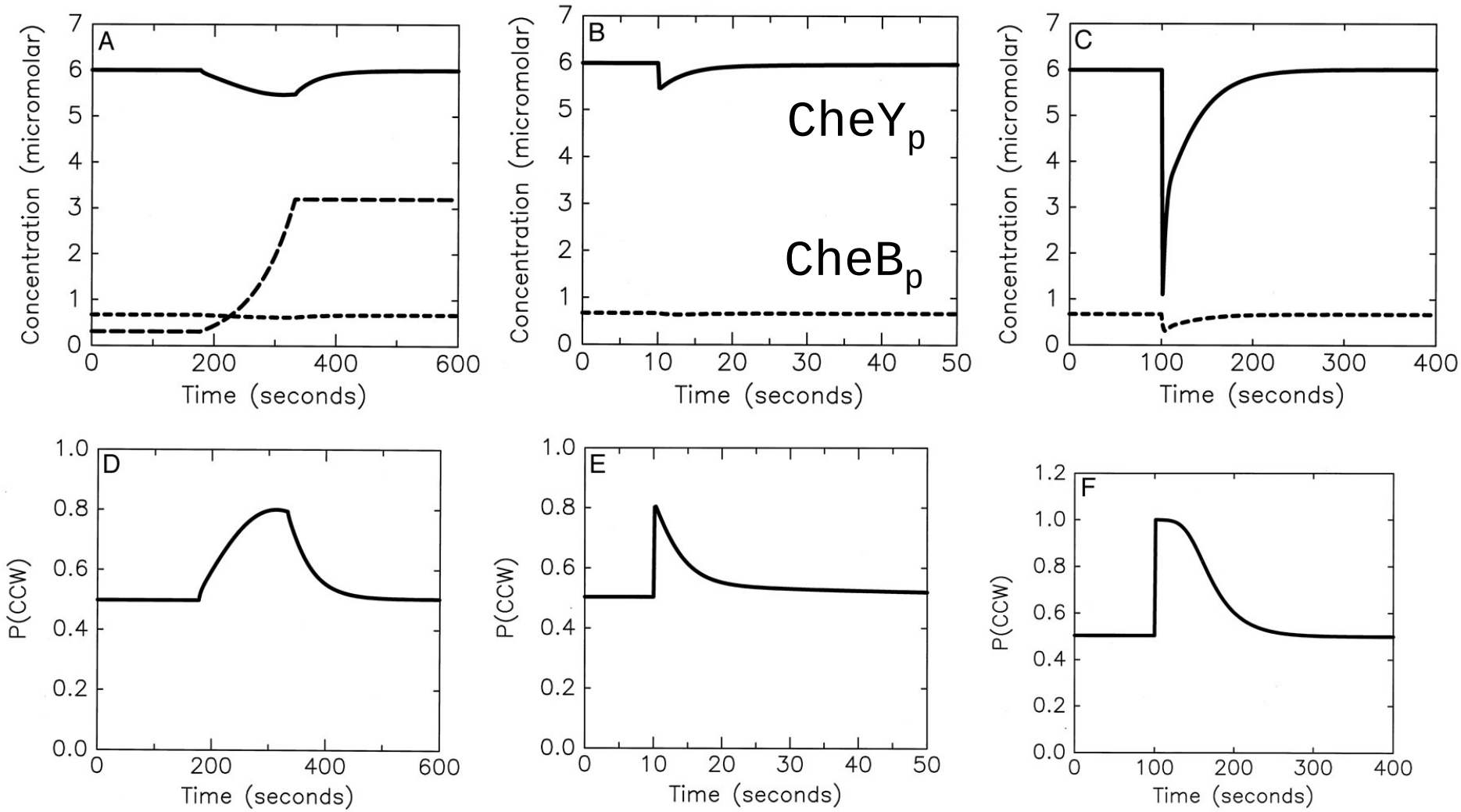
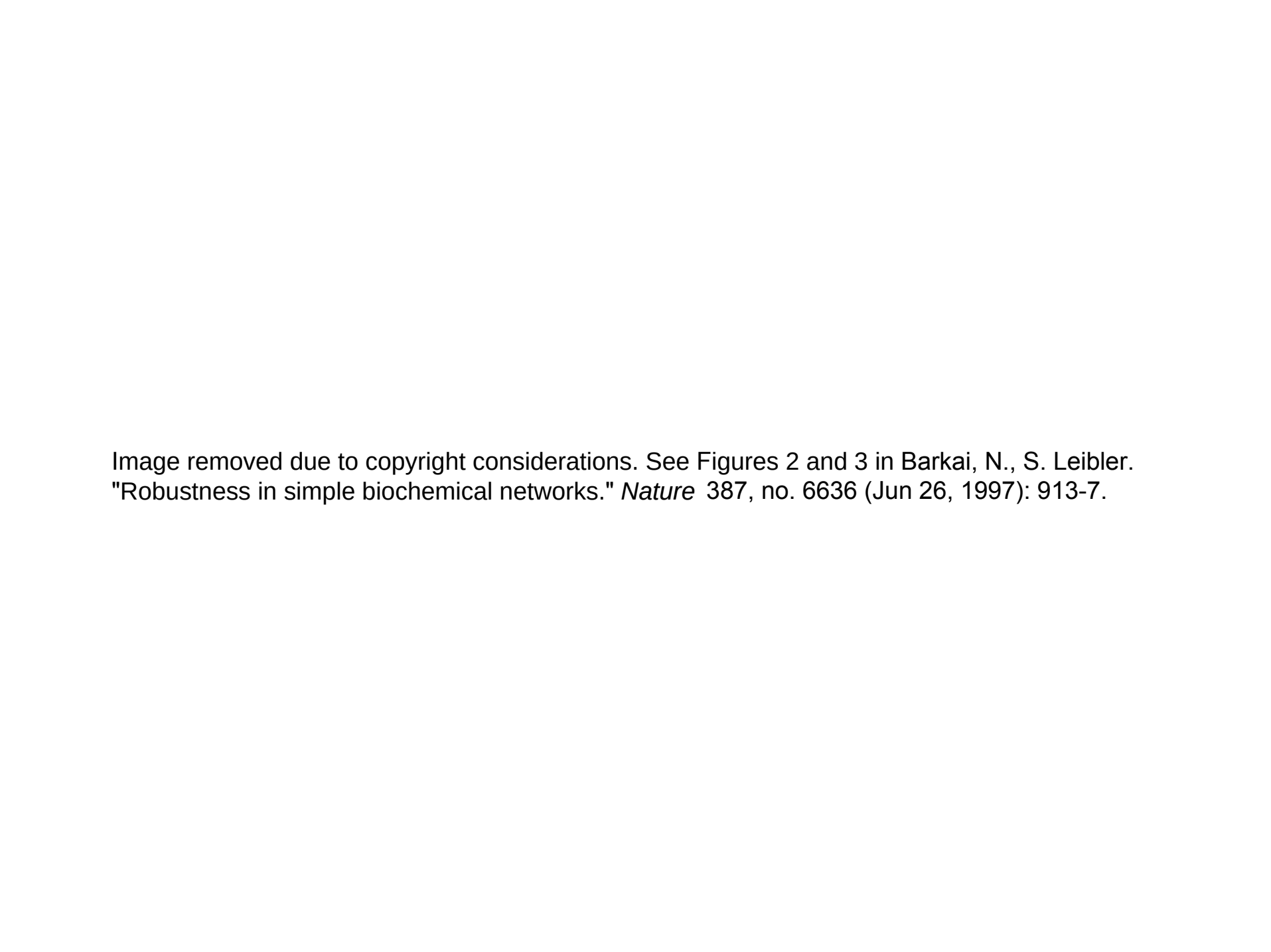


Figure 4 of Spiro P. A., J. S. Parkinson, and H. G. Othmer. "A model of excitation and adaptation in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 94, no. 14 (Jul 8, 1997): 7263-8.



Spiro, P. A., J. S. Parkinson, and H. G. Othmer. Figures 1, 2, and 4 in "A model of excitation and adaptation in bacterial chemotaxis." *Proc Natl Acad Sci U S A* 94, no. 14 (July 8, 1997): 7263-8.

Image removed due to copyright considerations. See Figures 2 and 3 in Barkai, N., S. Leibler. "Robustness in simple biochemical networks." *Nature* 387, no. 6636 (Jun 26, 1997): 913-7.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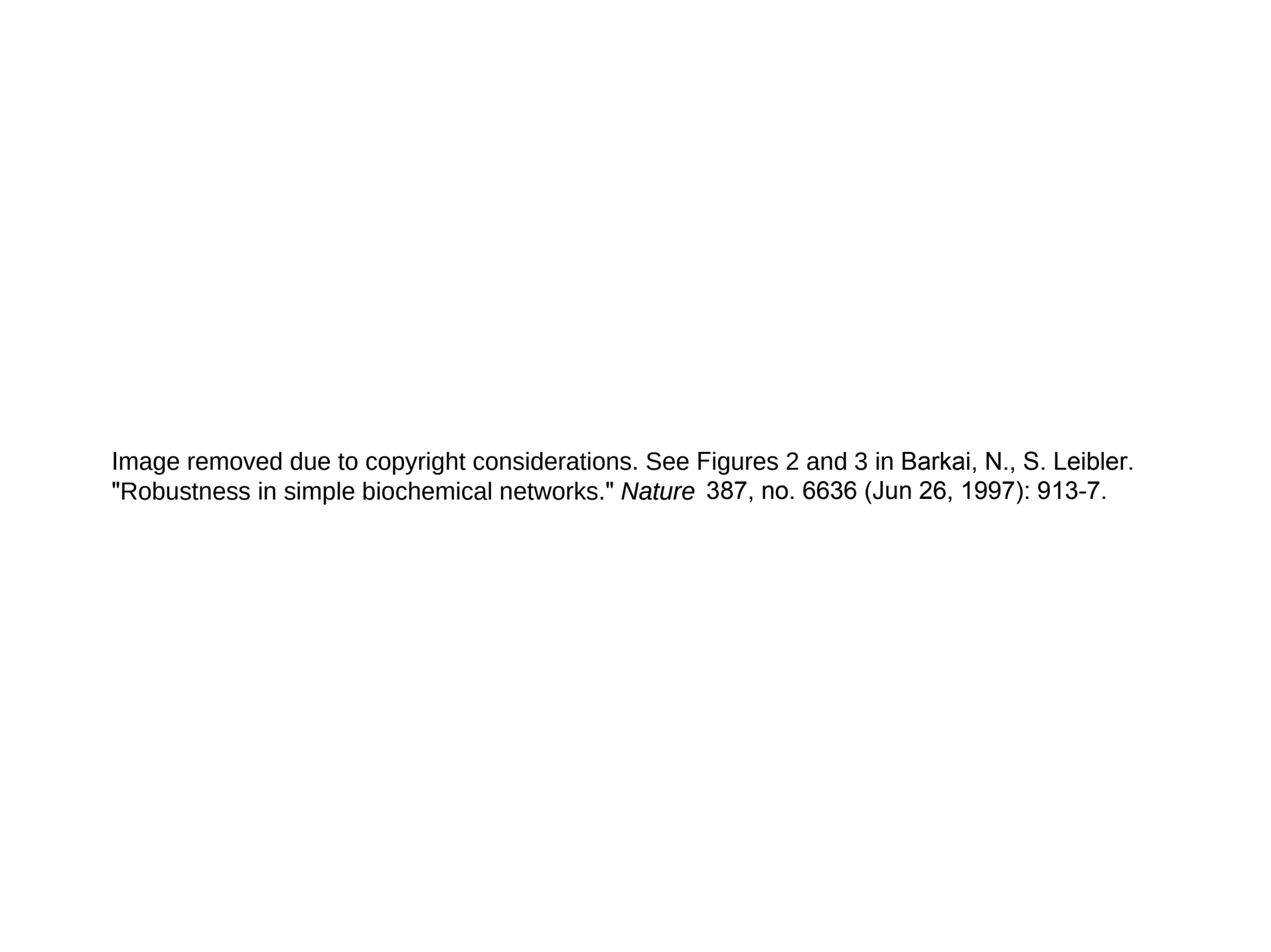
"Robustness in simple biochemical networks." *Nature* 387, no. 6636 (Jun 26, 1997): 913-7.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"Robustness in simple biochemical networks." *Nature* 387, no. 6636 (Jun 26, 1997): 913-7.