Part III

Small network, high performance

Bacteria chemotaxis

based on pioneered works by Howard Berg and others

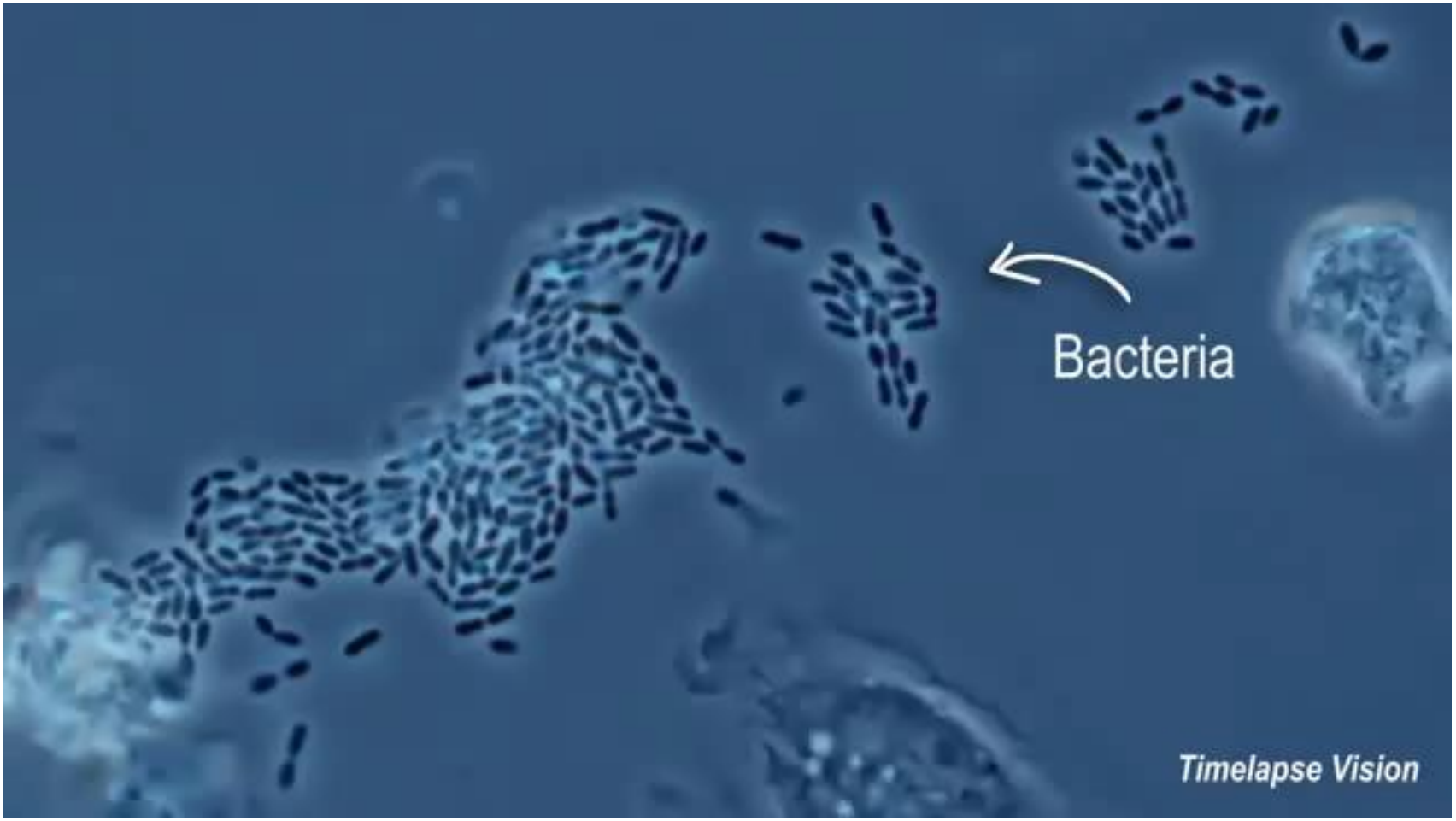
Week 5-6

Cell chemotaxis

- Chemotaxis (from *chemo-* + *taxis*) is movement of an organism in response to a chemical stimulus.
- Is precisely controlled in many developmental and physiological process
- The underline biochemical network has been gradually deciphered
- Correlation between biochemical reactions and precise control required systems biology approaches

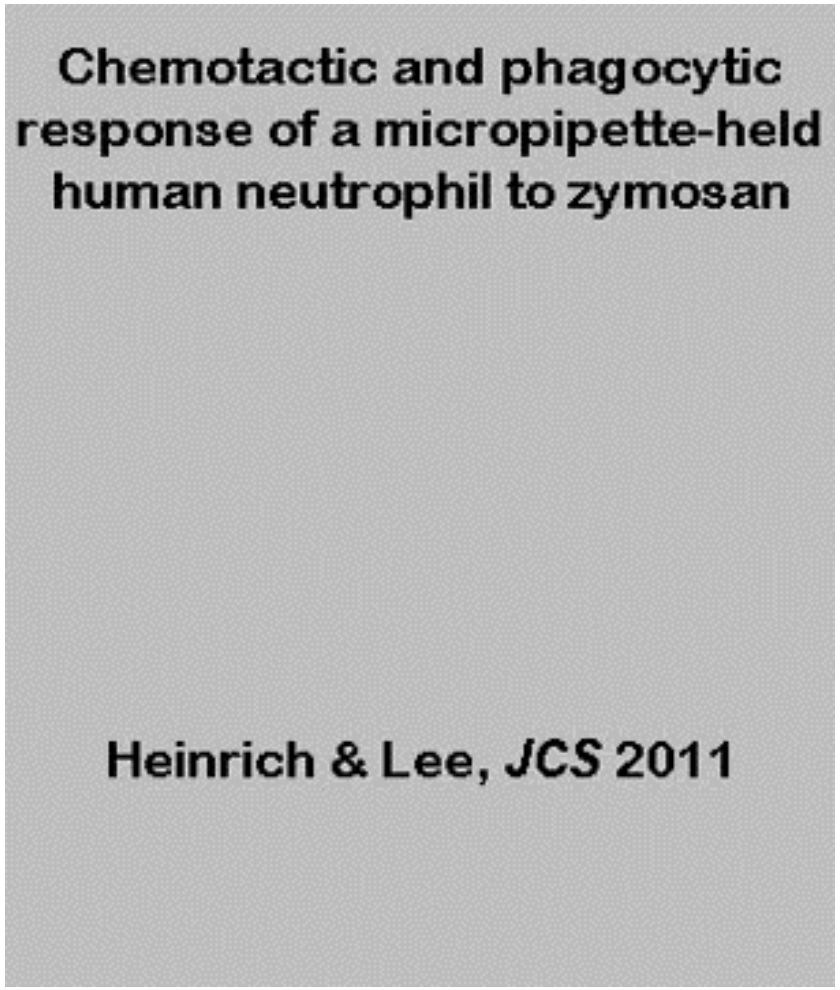
Examples of chemotaxis

1. Neutrophil： eating bacteria



Examples of chemotaxis

1. Neutrophil： how to find bacteria, fungi



Zymosan is a glucan from the surface of fungi

Examples of chemotaxis

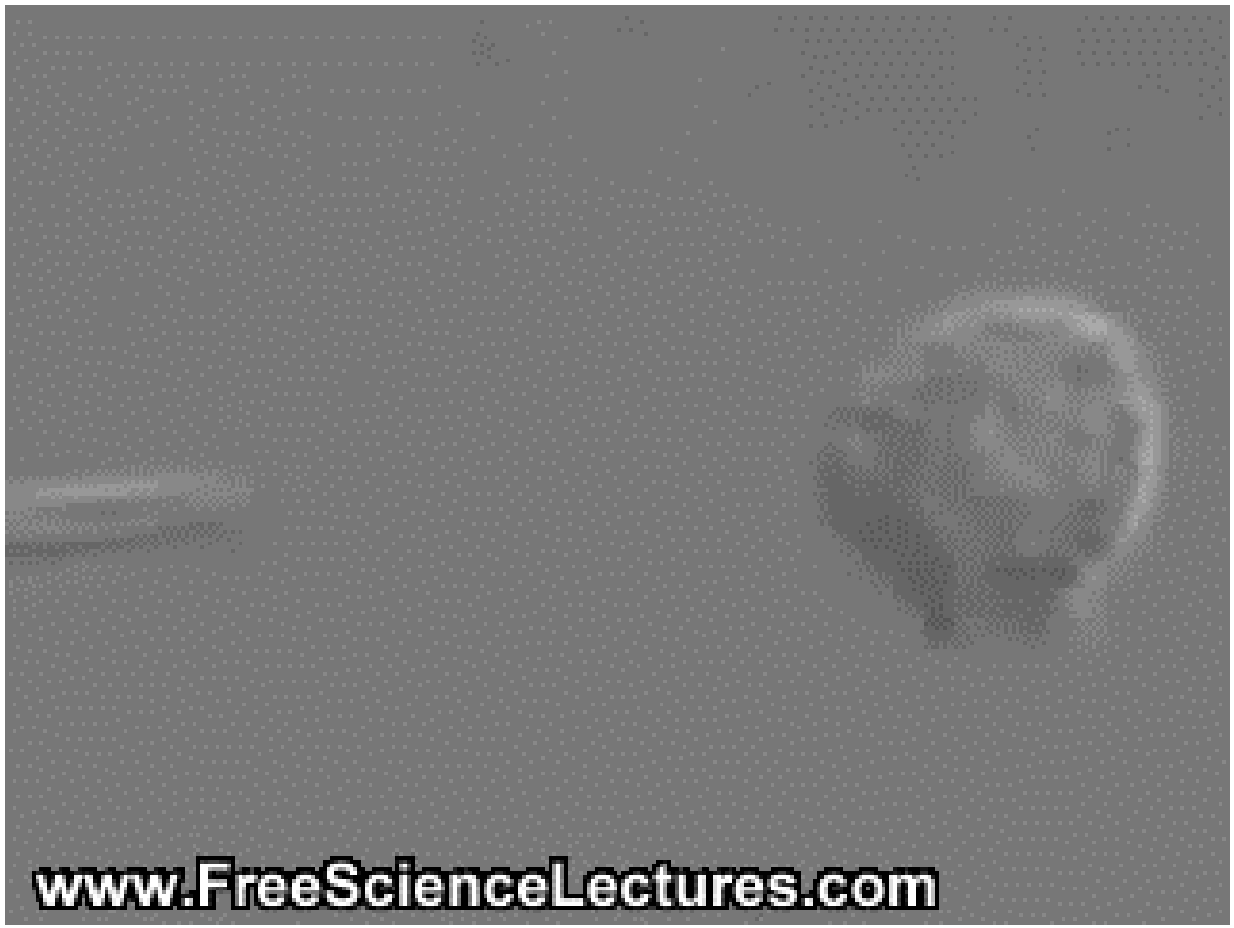
1. Neutrophil: how to find bacteria, fungi

Neutrophil chasing bacterium

Sensing LPS produced by bacteria

Examples of chemotaxis

1. Neutrophil: how to call for reinforcement? Secreting chemokines that attract other neutrophils

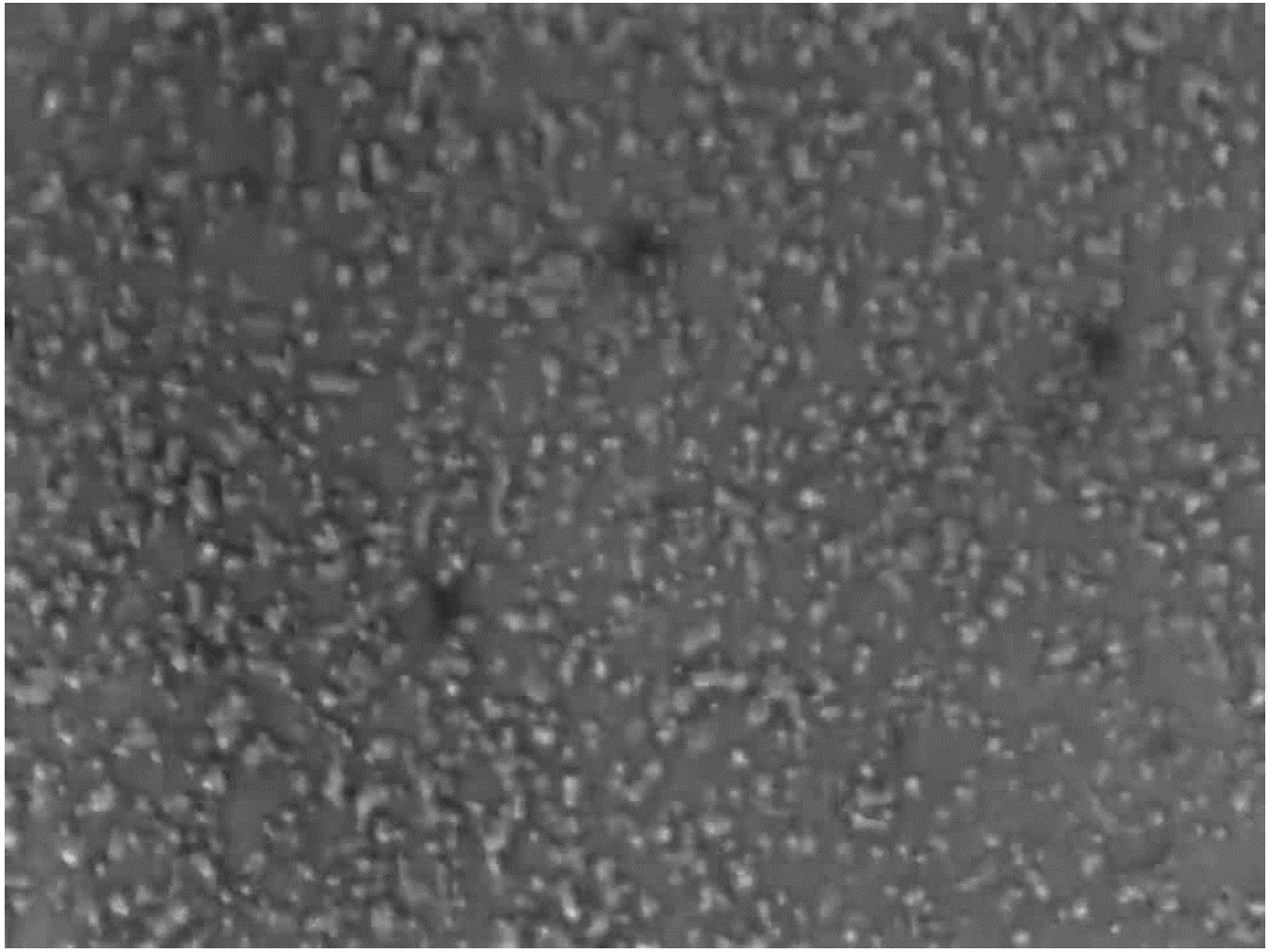


Examples of chemotaxis

2. Dictyostelium: moving towards high concentration of cAMP

Examples of chemotaxis

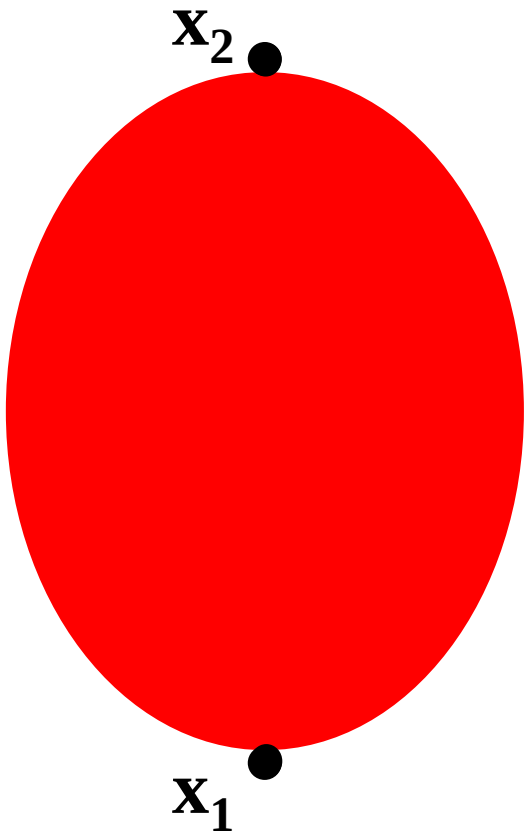
2. Dictyostelium: what does it for?



Examples of chemotaxis

2. Dictyostelium: what does it for?

How do they find the direction of high concentration chemoattractants?



Spatial Sensing

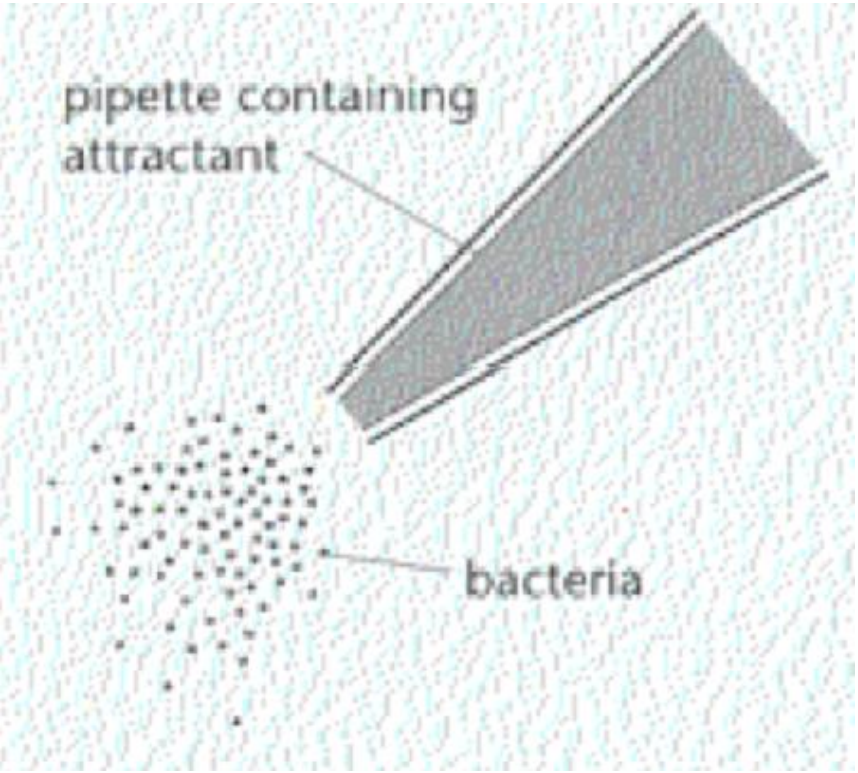
$$\frac{C(x_2)-C(x_1)}{x_2-x_1}=\frac{dC}{dx}$$

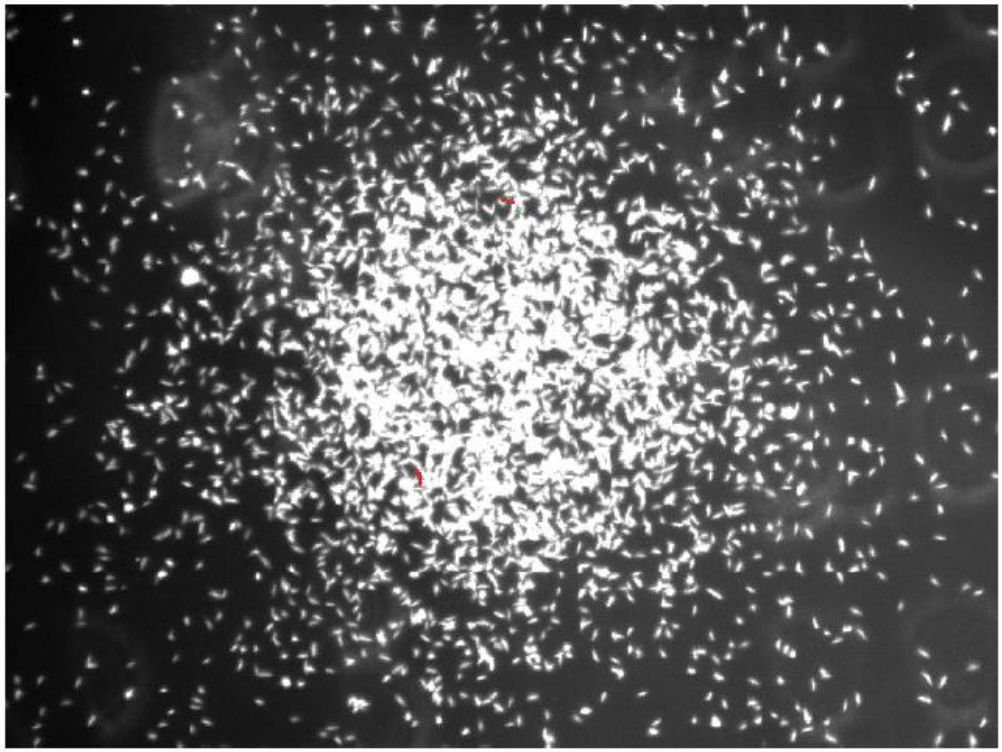
if $\frac{dC(x_2)}{dx}>0$, shmoo in direction of x_2

Challenge: how to find it in shallow gradient:
~1% difference between front and back?

An biochemically simplest example of chemotaxis

Bacteria finding food (e.g. aspartate,glucose) in the wild





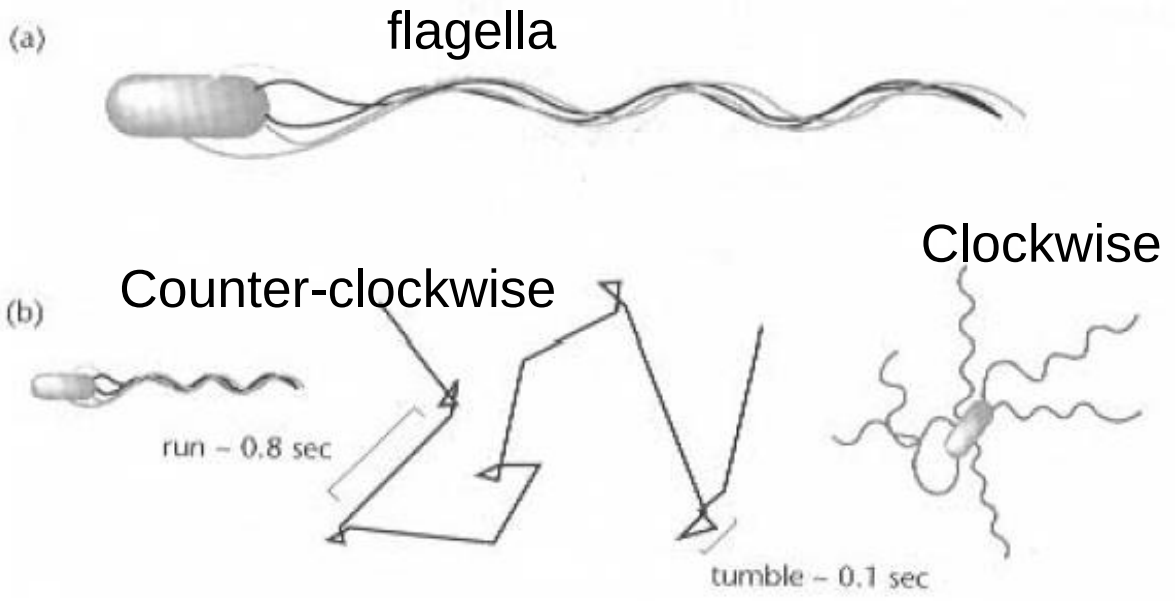
The length of a bacteria (~1μm) is much smaller than eukaryotic cell (~10μm), spatial sensing of gradient is even harder.

How does it achieve this?

Bacteria movement

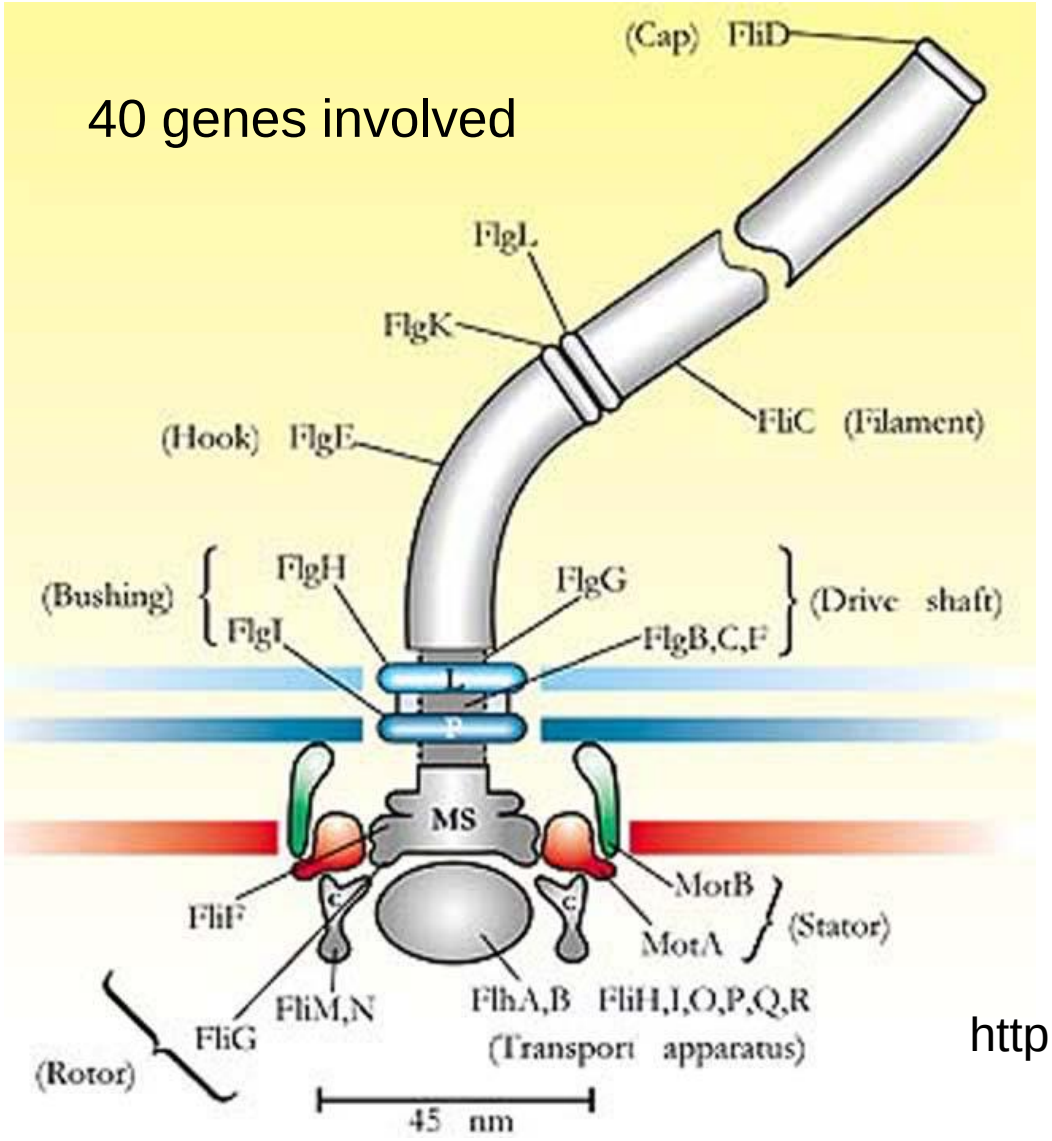
When swimming, the cell travels in a straight line for about 1sec, travels about 30μm. When tumbling, the cell changes on average by 60 degrees.

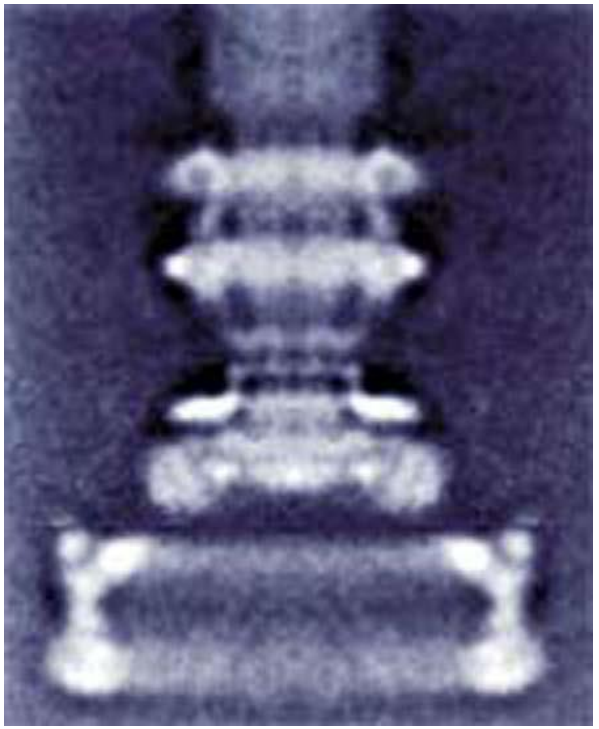
cell length ~ 3 μm, diameter ~ 1 μm



Flagella is labeled with conjugated fluorescent dye, and imaged with high speed fluorescent microscopy.
Turner and Berg, 2000

Structure of the flagella machinery

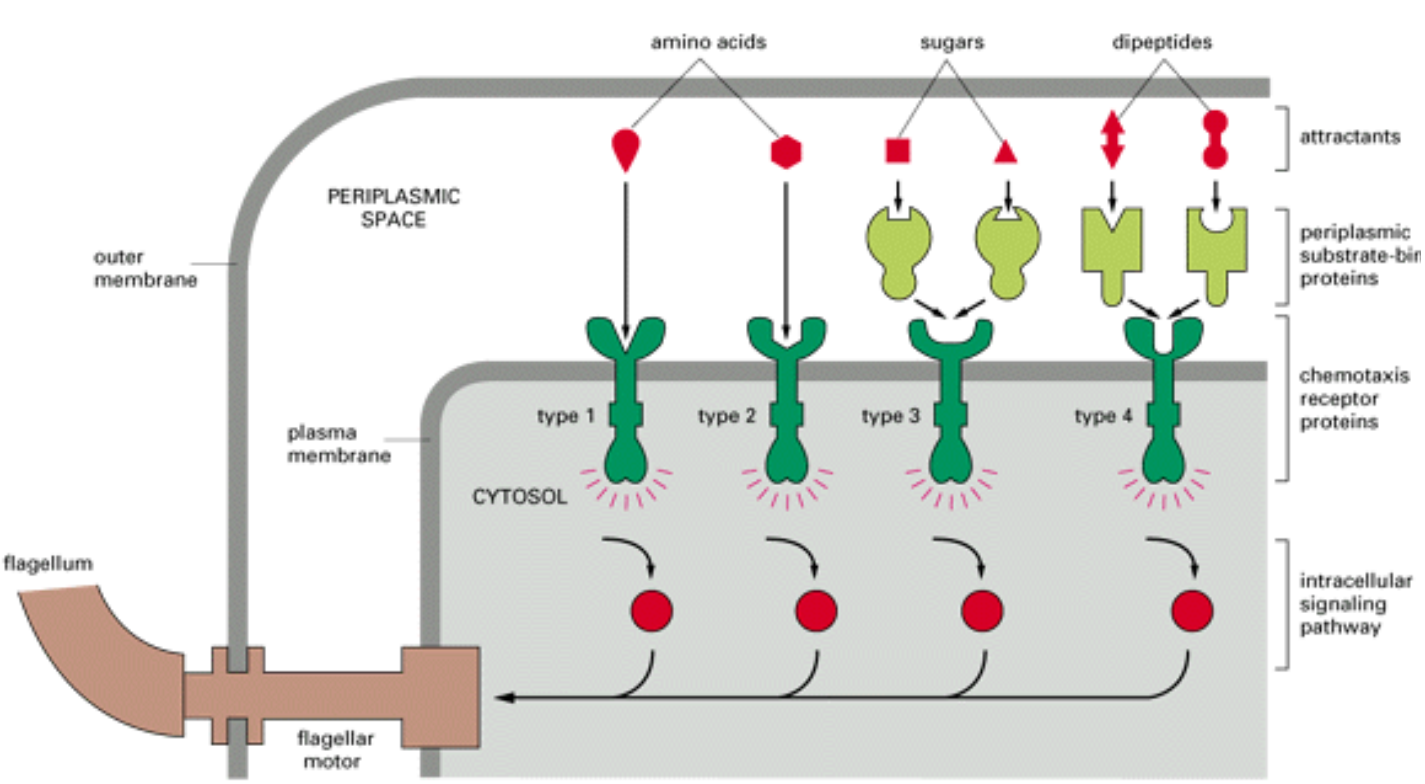


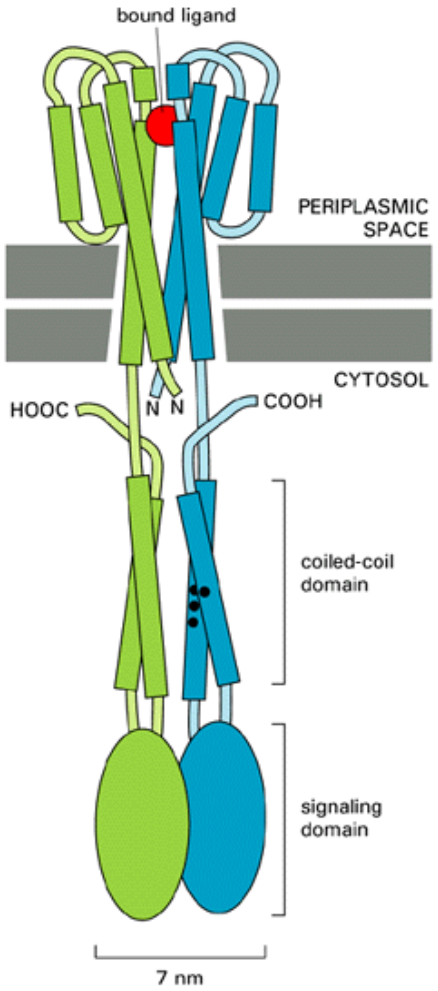


http://www.aip.org.pt/jan00/berg.htm

Rotary 8 cylinder motor driven by protonmotive force (400 steps/rev)

Receptors sensing chemoattractants



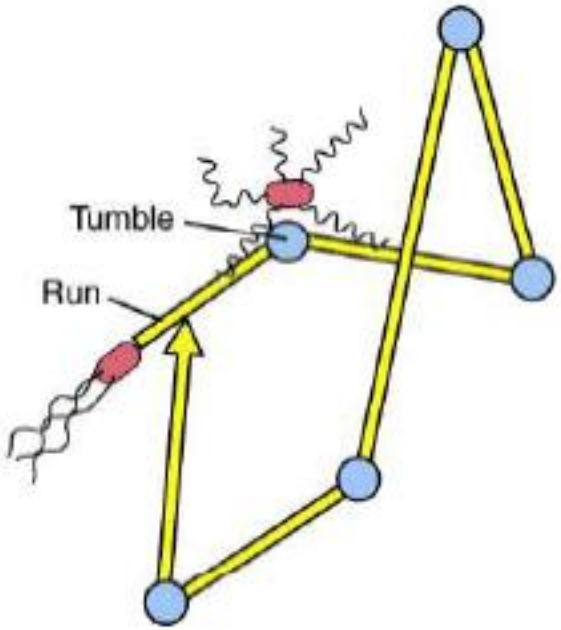


Alberts et al. Mol Biol Cell 3rd Ed.

Classes of Chemoreceptors

- Originally called methyl-accepting chemotaxis proteins (MCPs)
- Four Types:
  - tsr (MCP I)
  - tar (MCP II) (important, binds aspartate)
  - trg (MCP III)
  - tap (MCP IV)
- Mainly differ in periplasmic domain

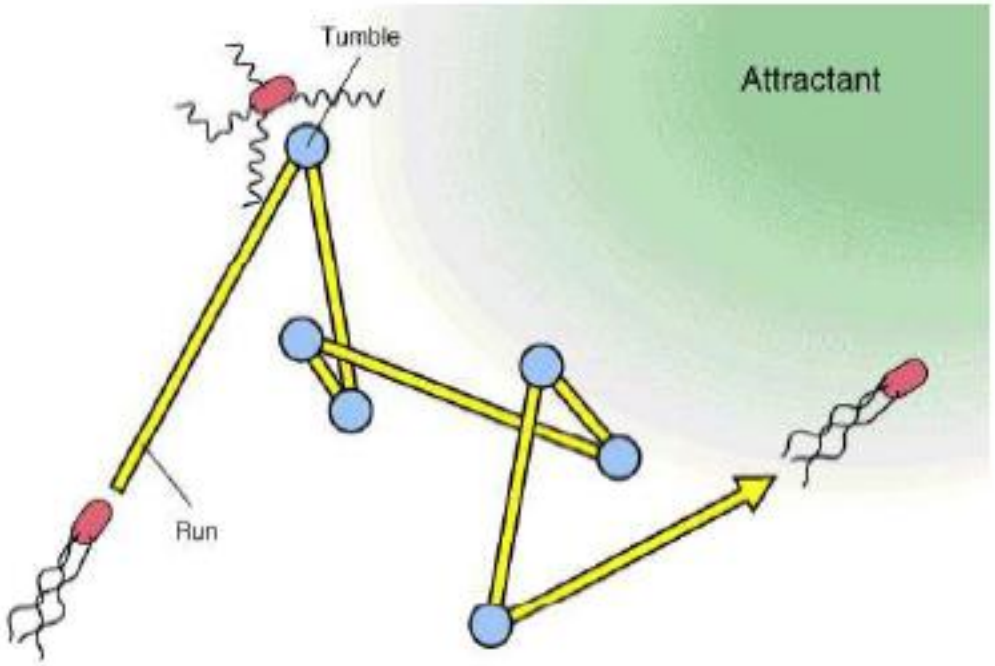
Absence of chemical attractant



Since bacteria cannot move straight and is too small to directly sense the spatial gradient, how does it perform chemotaxis?

The higher the attractant concentration, the lower probability of tumbling.

Presence of chemical attractant



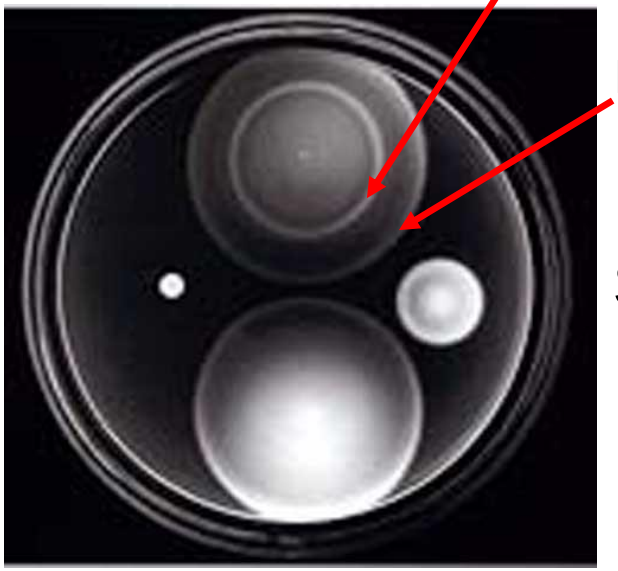
chemical gradient sensed in a temporal manner

Mutational approaches to tease out genes mediating chemotaxis

Plate contains serine and aspartate

Looking for Asp (2nd choice)

Wild type



Looking for Ser (1st choice)

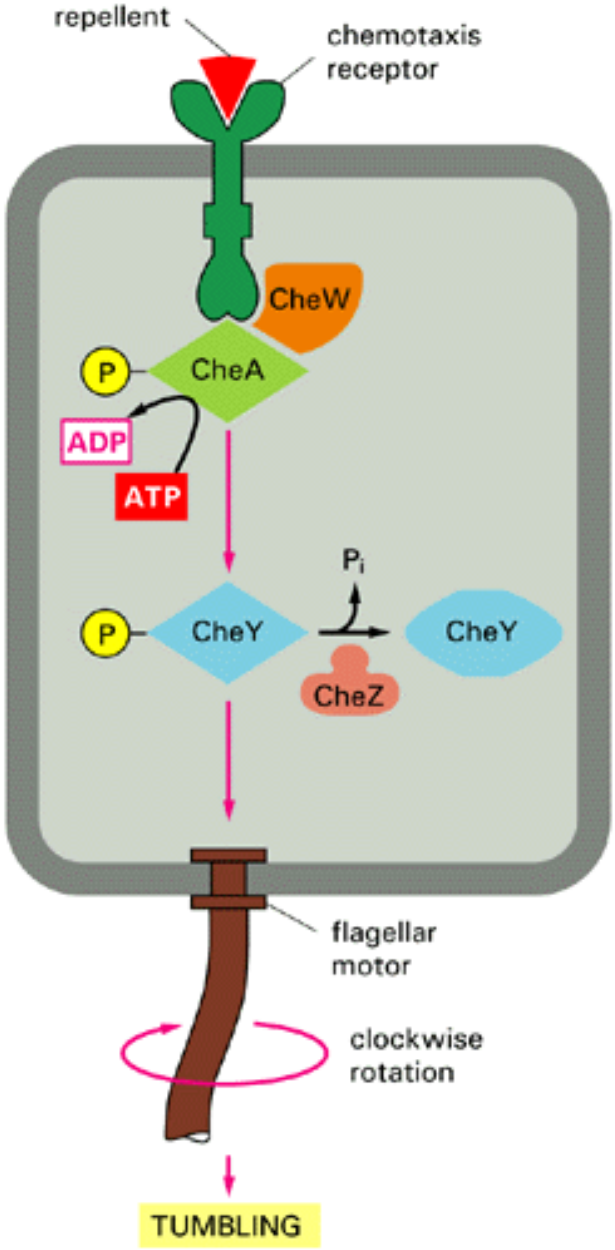
Non-motile *flg*

Ser detection$^-$ *tsr*

Asp detection$^-$ *tar*

http://www.aip.org.pt/jan00/berg.htm

The biochemical network performing the task



Counter-Clockwise = straight swim, bundled flagella

Clockwise = tumble, separate flagella

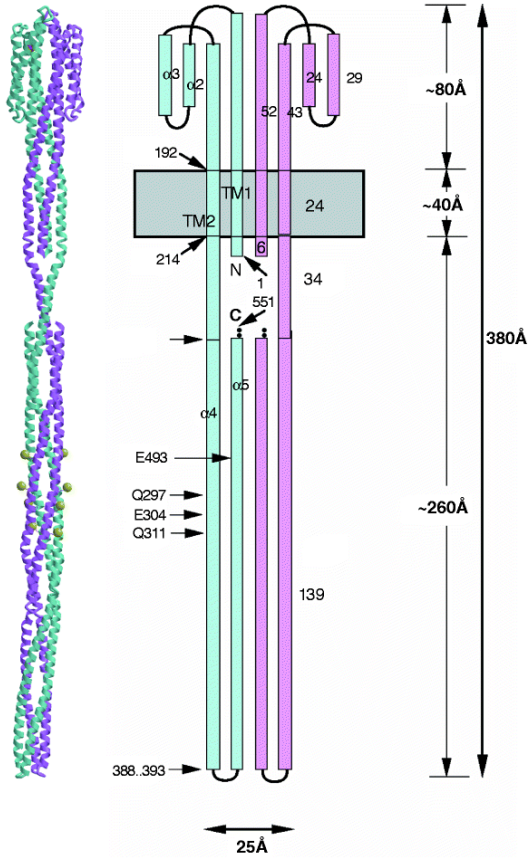
CheY-P binding to motor -> CW rotation

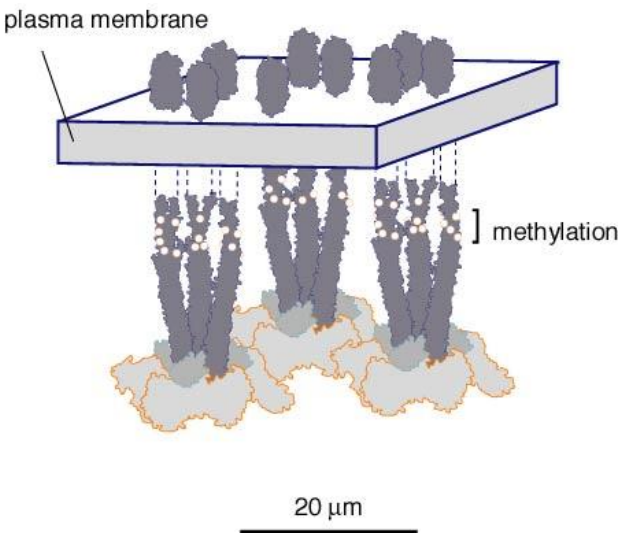
↑Repellent -> ↑CheA-P -> ↑CheY-P -> ↑ CW

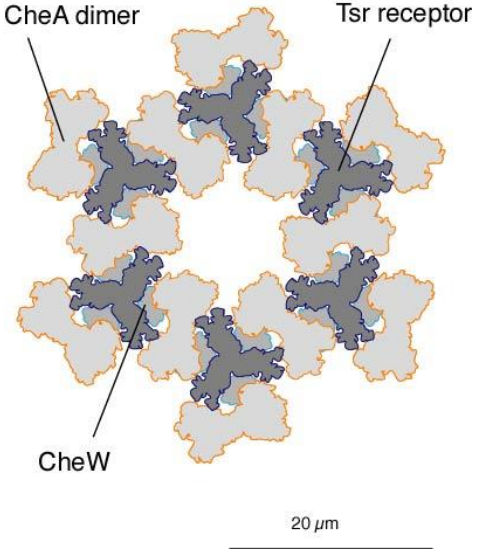
↑Attractant -> ↓CheA-P -> ↓CheY-P -> ↓CW

Alberts et al. Mol Biol Cell 3rd Ed.

The spatial organization of chemotaxis receptors

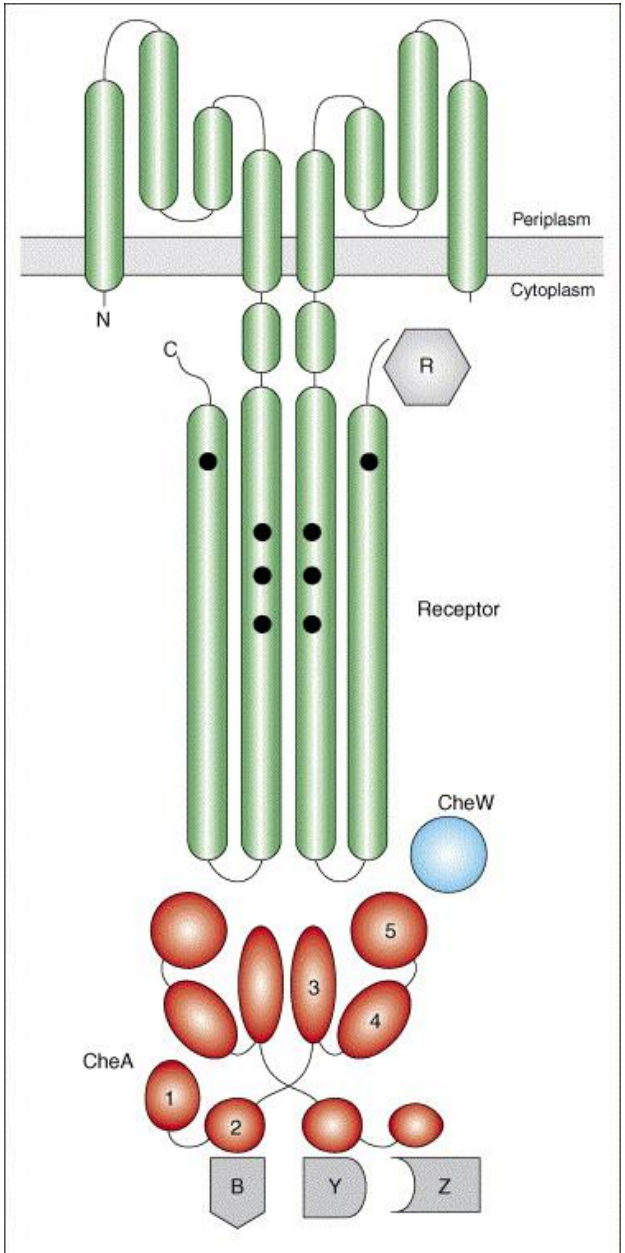




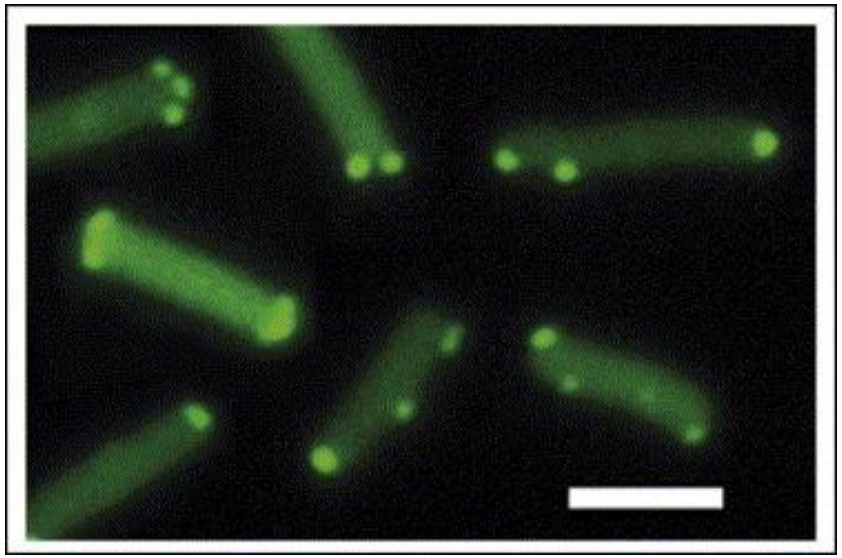


http://www.pdn.cam.ac.uk/groups/comp-cell/Research.html

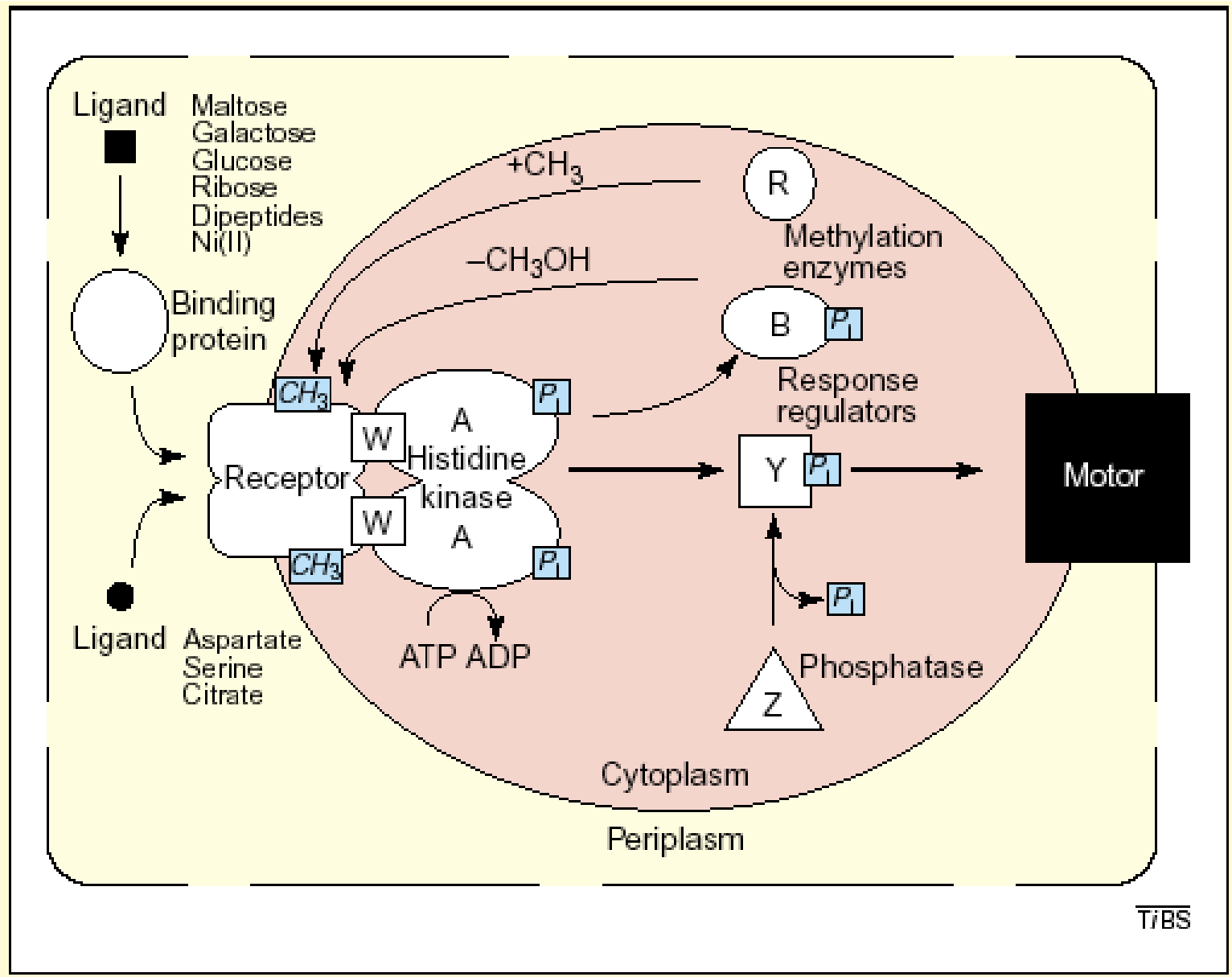
Receptor Localization



Kentner and Sourjik

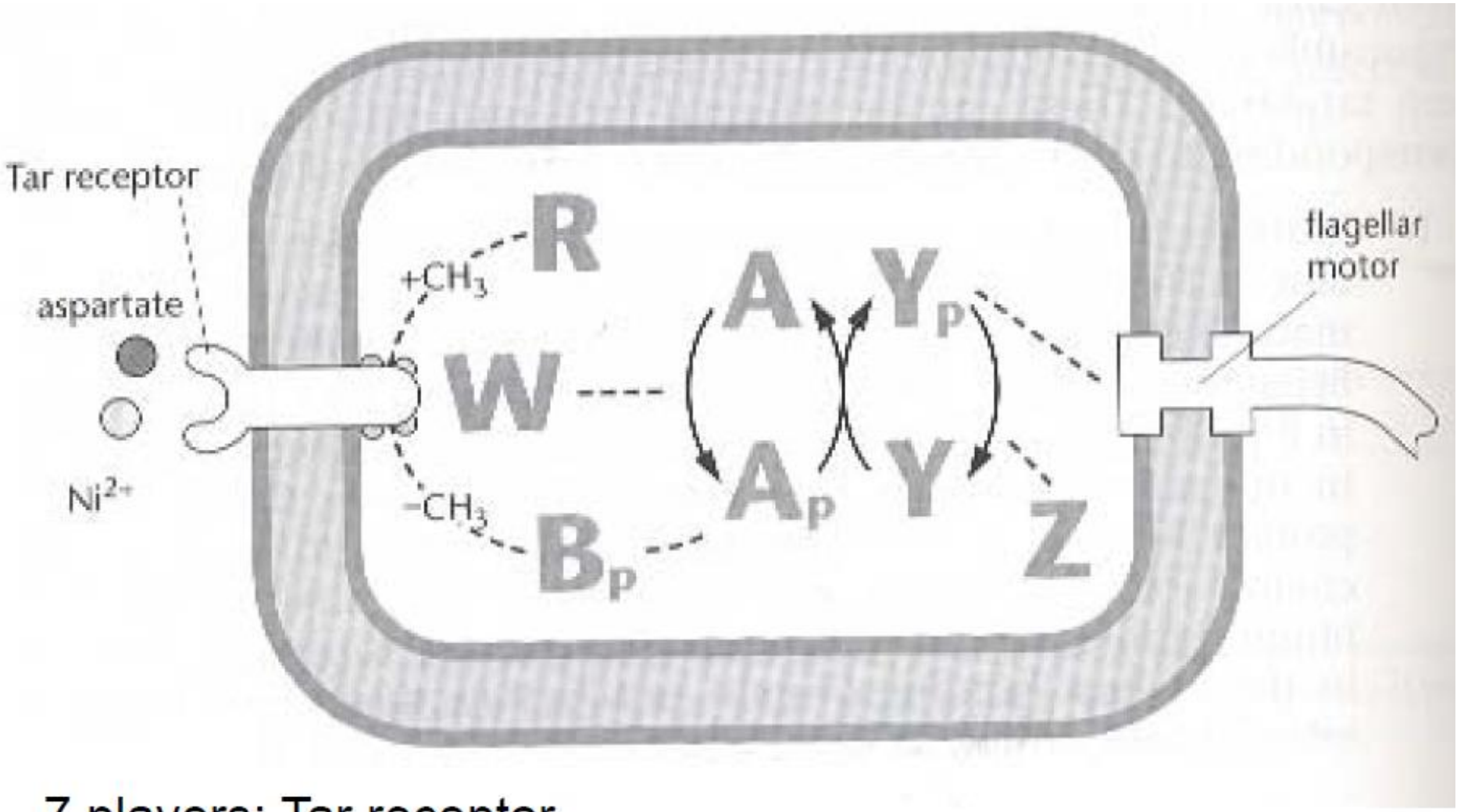


The entire chemotactic network



Falk and Hazelbauer (2001) TIBS 26, 257

Simplified drawing of the chemotactic network

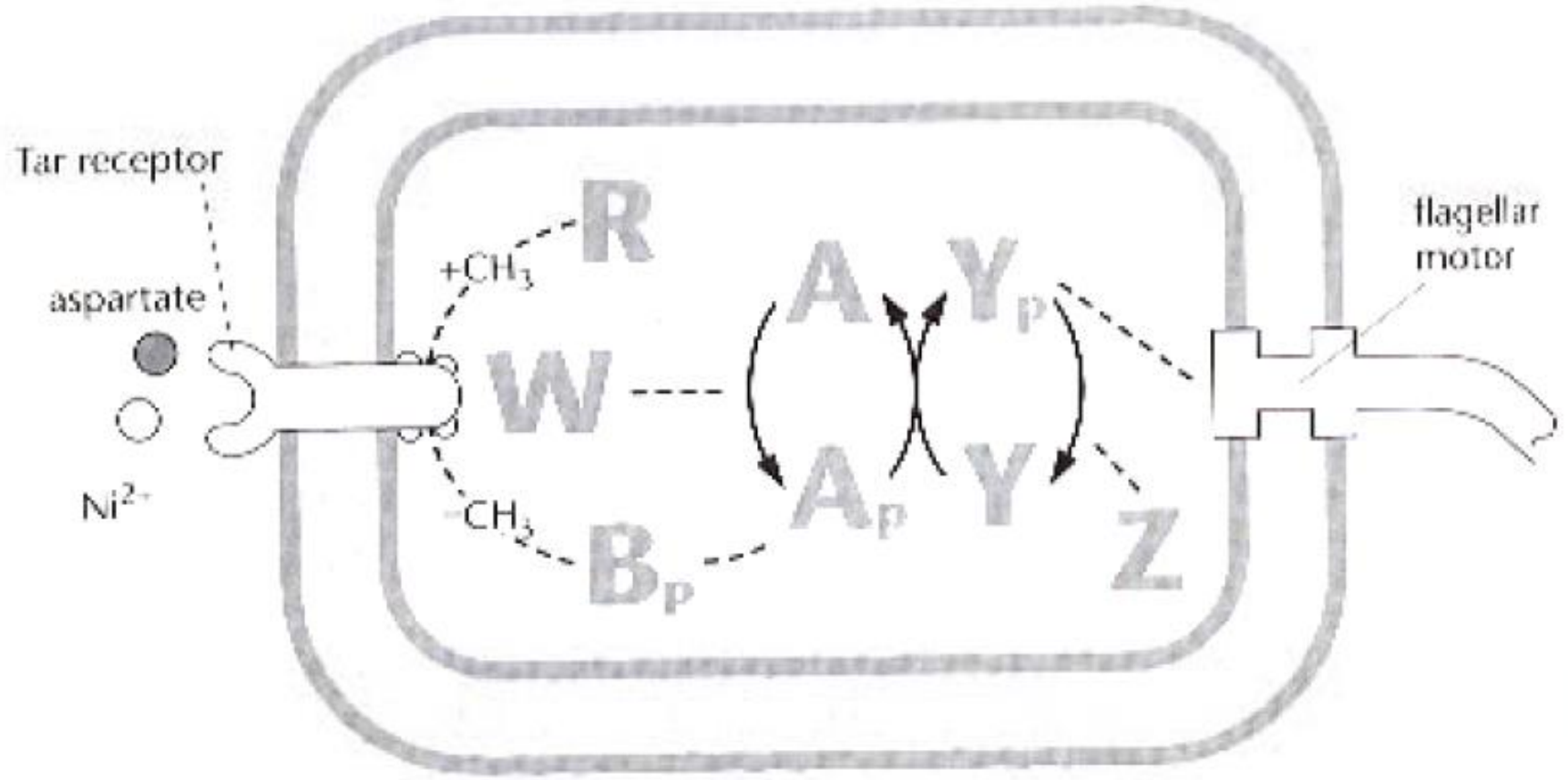


7 players: Tar receptor,
CheA, CheB, CheR, CheW, CheY, CheZ

Proteins in the network can be post-translational modified in different ways

I Phosphorylation (CheA, CheY, CheB)

II Methylation (Tar receptor)



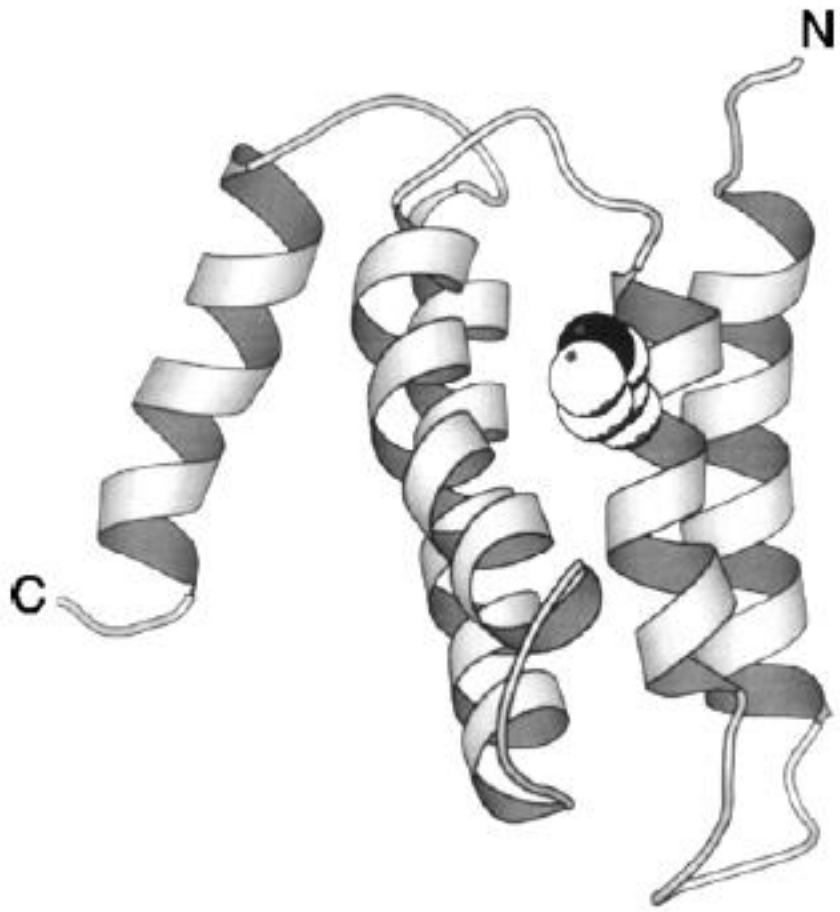
I Phosphorylation (CheA, CheY, CheB)

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

$$\mathrm{CheA} + \mathrm{ATP} \leftrightarrow \mathrm{CheA}_{\mathrm{p}} + \mathrm{ADP}$$

CheA (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')

CheA$_p$ is unstable and transfers its phosphoryl group to CheY (highly soluble, diffuses through the cytoplasm



CheA
His48

Falke et al. Annu. Rev. Cell Dev. Biol. **13,** 457 (1997)

I Phosphorylation (CheA, CheY, CheB)

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

phosphoryltransfer: $\mathrm{CheA}_{\mathrm{p}} + \mathrm{CheY} \leftrightarrow \mathrm{CheA} + \mathrm{CheY}_{\mathrm{p}}$

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

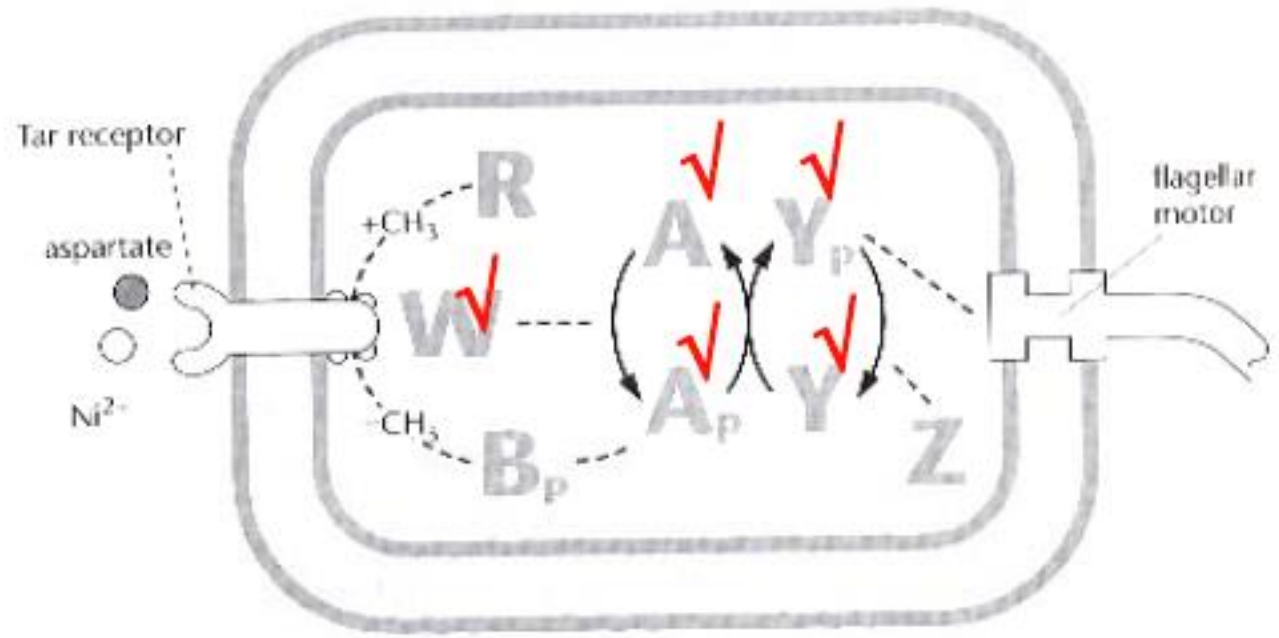
logic:

high levels of CheAp -> high levels of CheYp

(lots of tumbles)

low levels of CheAp -> low levels of CheYp

(straight swimming)



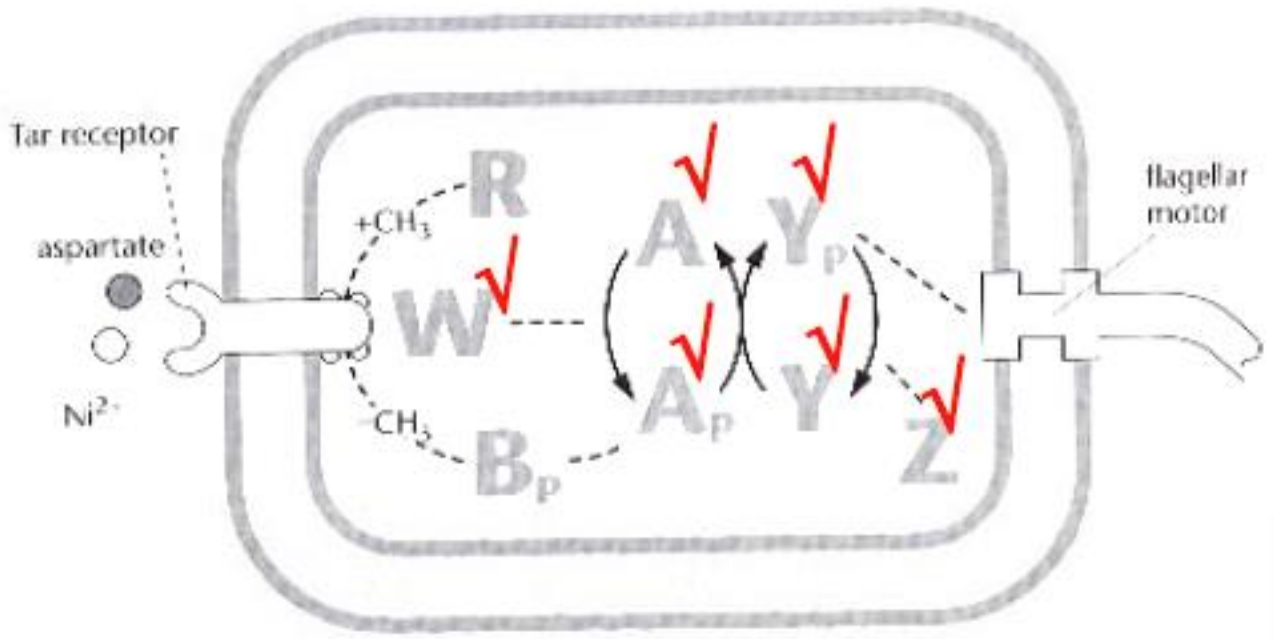
CheZ dephosphorylates $CheY_p$
(phosphatase, opposite function as CheA)

$$CheY_p + CheZ \rightarrow CheY + CheZ$$

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

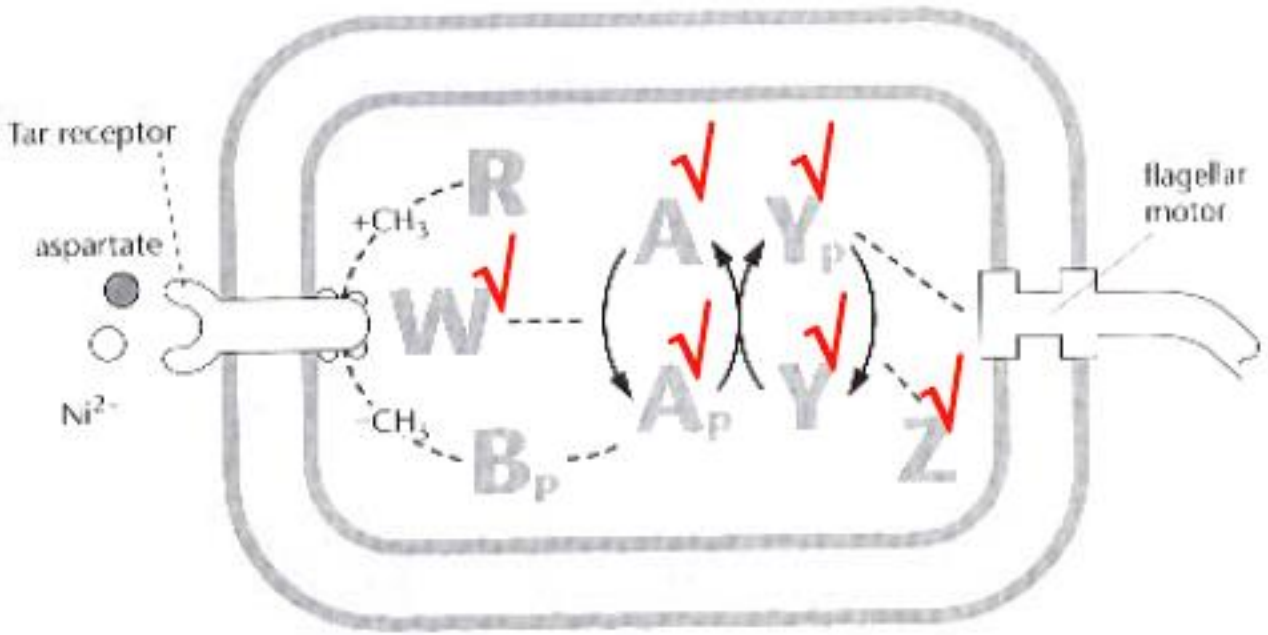
(straight swimming)

II Methylation (tar receptor)



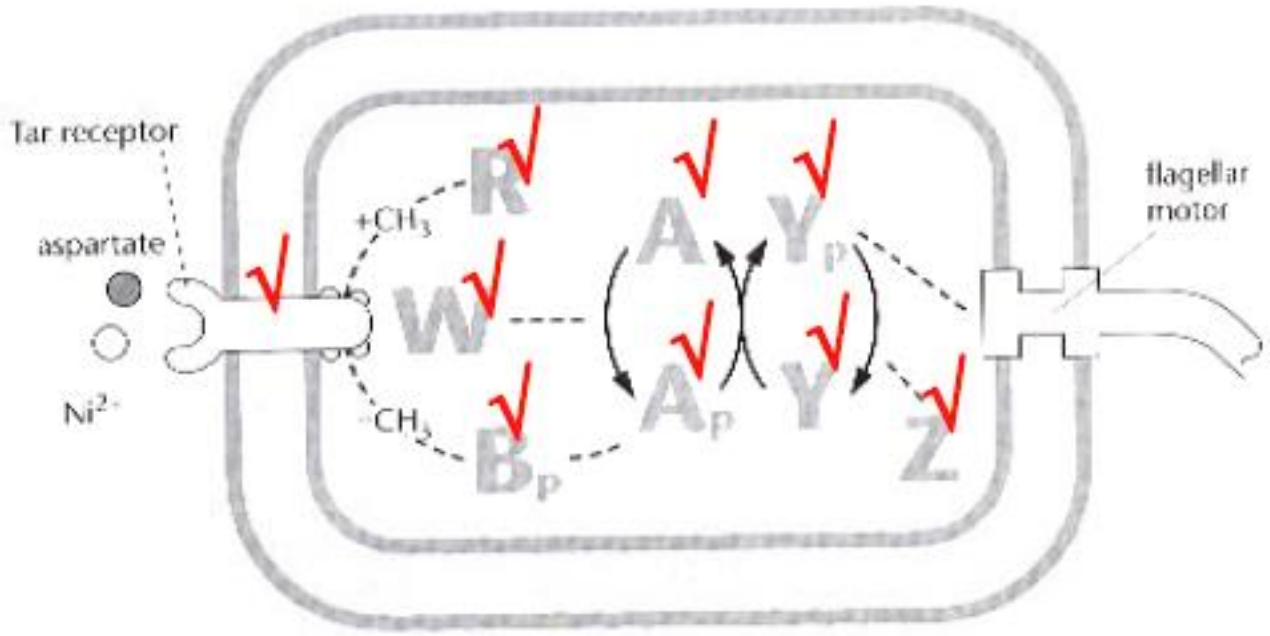
CheR adds methyl group
CheB$_p$ removes methyl group

Methylation - Phosphorylation coupling



phosphorylation state of CheB is controlled by CheA

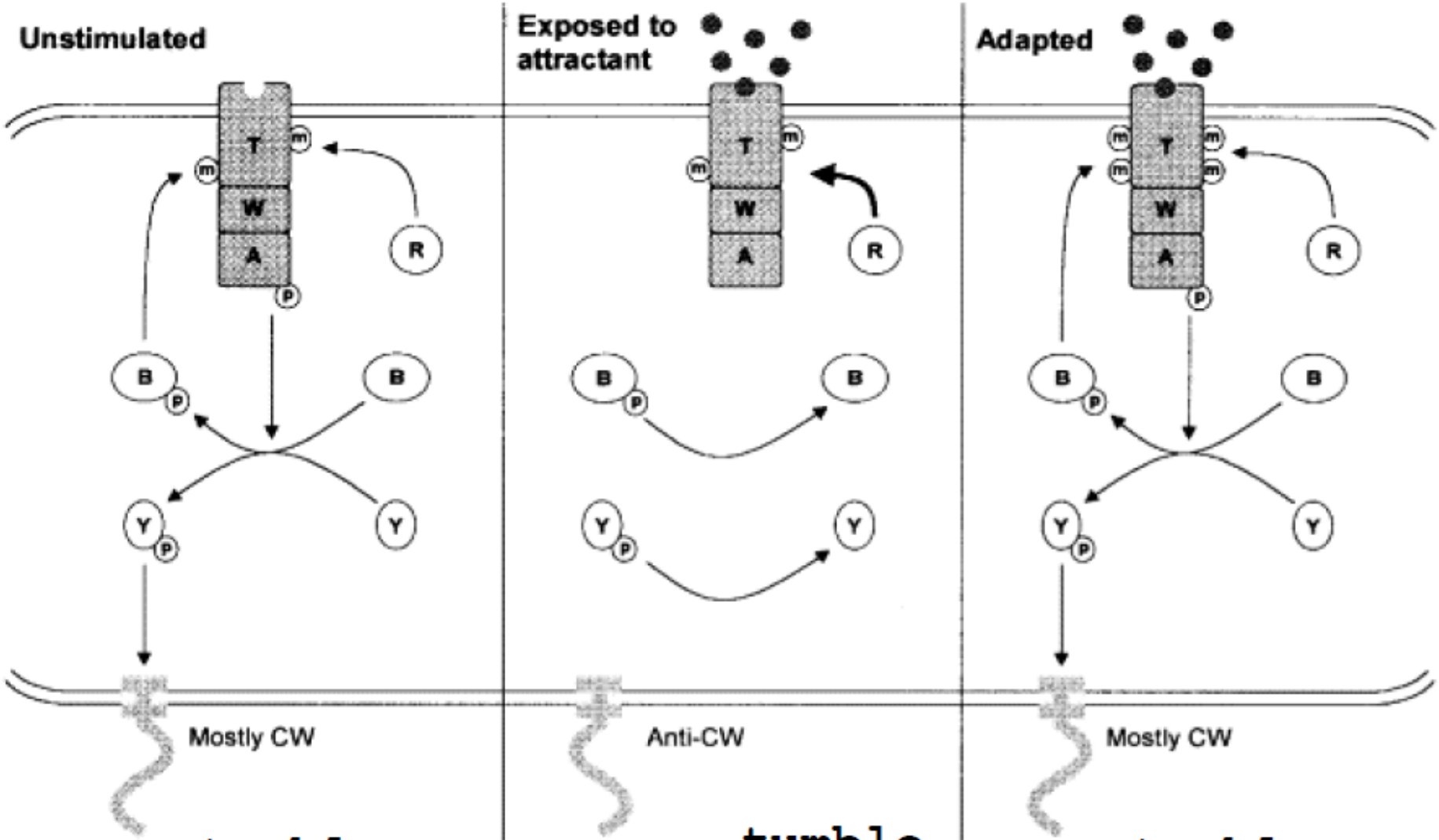
Role of ligand binding



The rate of CheA phosphorylation is stimulated by unoccupied receptors

Competing process of different time scale leads to the critical adaptation

- The attractant/repellant is detected by the chemoreceptors on the cell surface
- Signal is sent to a central processing system
- Central processing system signals the flagellar motor - Excitation (fast)
- The system adapts to the ligand concentration in the environment outside the cell - Adaptation (slow)

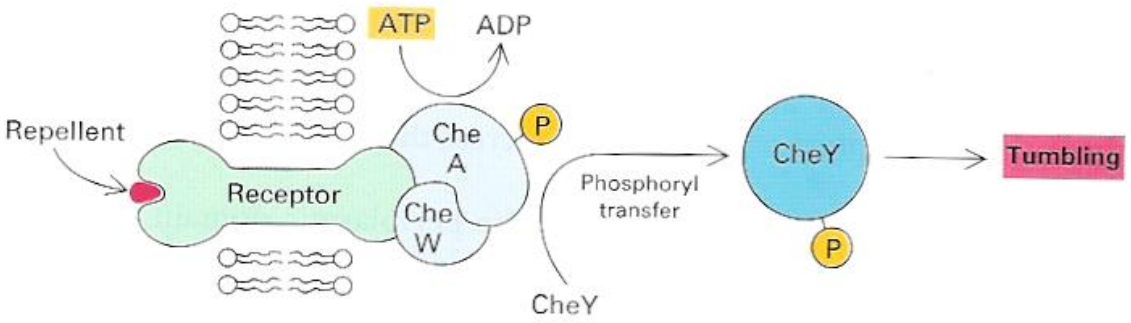

Unstimulated
Exposed to attractant
Adapted
T
W
A
R
B
Y
m
P
Mostly CW
Anti-CW
Mostly CW
tumble
tumble
suppression
tumble

Fast Response - Excitation

- Normally, CheA autophosphorylates slowly on a histidine residue
  - CheA bound with CheW and receptor
  - Unoccupied receptor or repellant-receptor complex increases autophosphorylation
  - Attractant-receptor complex does not
- A phosphate group can be transferred to an aspartate residue on CheY

Fast Response - Excitation

- CheY ultimately determines direction of rotation
  - Phosphorylated CheY (CheYp) induces tumbling, unphosphorylated CheY does not
  - CheYp is dephosphorylated by the phosphatase CheZ

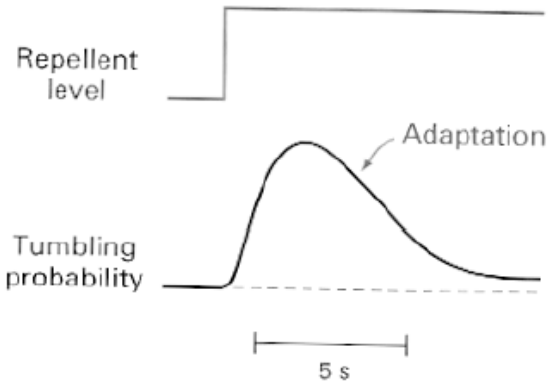


Fast Response - Excitation

- More attractant → less CheAp → less CheYp → less tumbling
- Less attractant/More repellant → more CheAp → more CheYp → more tumbling

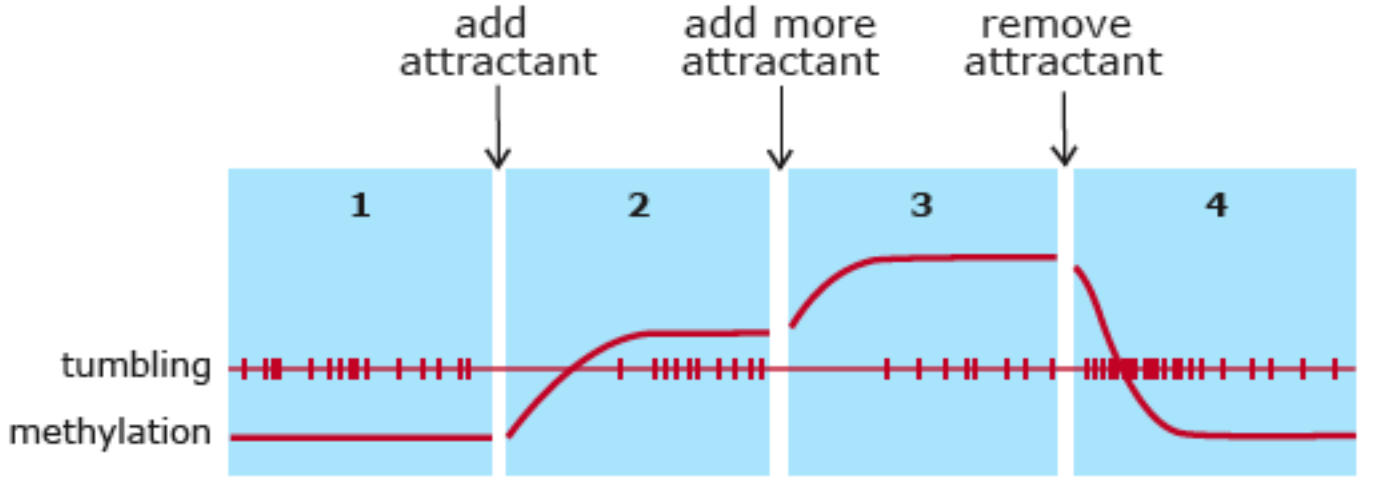
Slow Response - Adaptation

- After an increase in stimuli, system eventually returns to normal tumbling probability
- Adaptation controlled by reversible methylation of chemoreceptors - negative feedback
  - CheR is a methyltransferase (adds methyl)
  - CheB is a methylesterase (removes methyl)



Why does it have to be so complex?

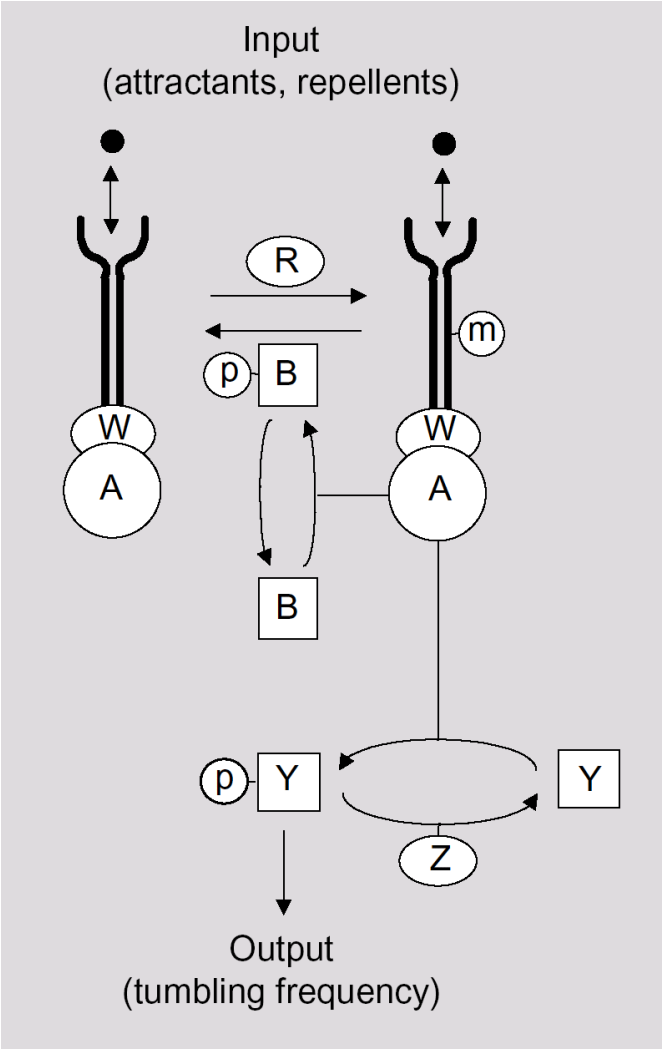
Exact Adaptation and sensitivity

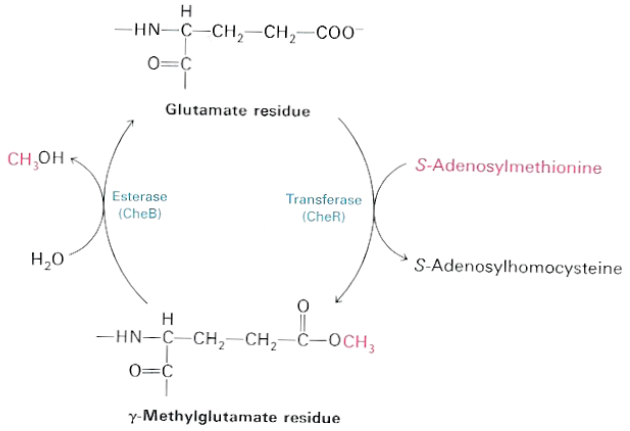


Correlation of Receptor Methylation with Behavioral Response

- Methylation is important for adaptation
- E. coli can sense aspartate from **10nM-1mM**
- Sense gradients as shallow as **0.1%**

CheB/CheR Methylation and Adaptation

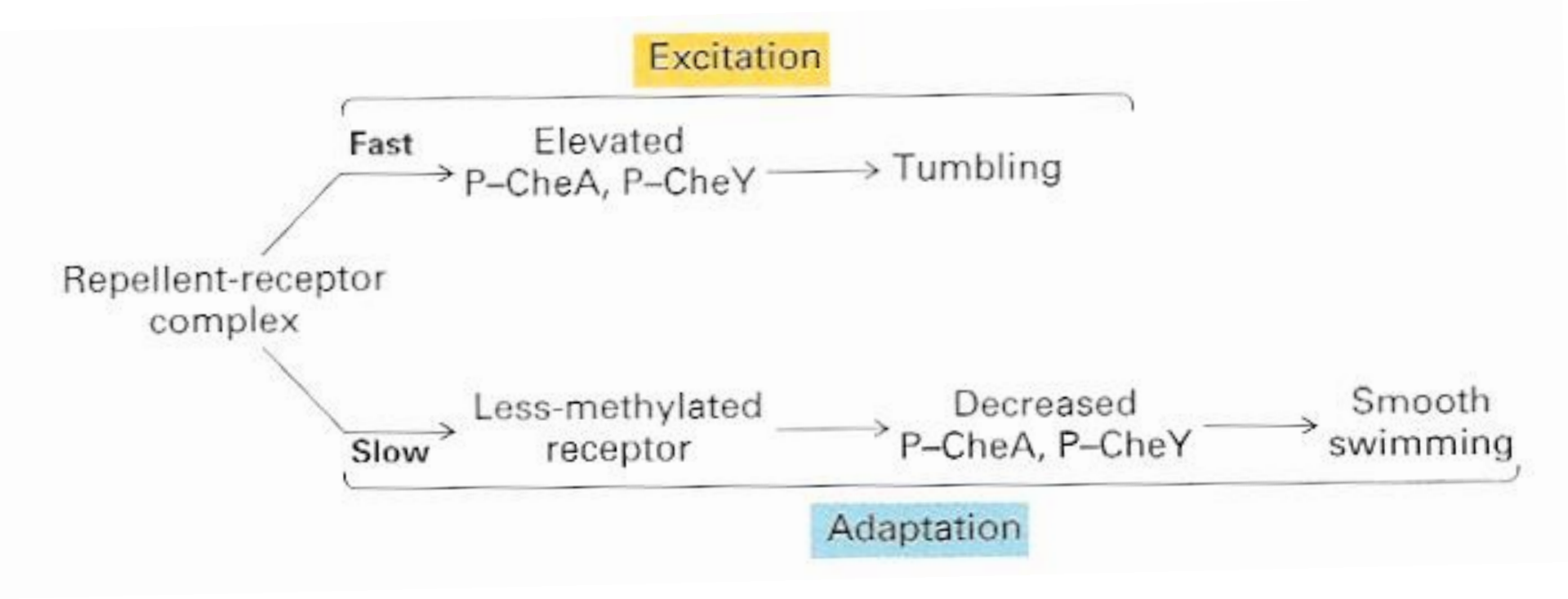




1) CheR adds methyl group, CheB removes it
2) Attractant/receptor binding → methylation up
   Repellent/receptor binding → methylation down
3) Methylation → CheA auto-phosphorylation up
4) CheA a-p → CheY phosphorylation up
   CheA a-p → CheB phosphorylation up
5) CheB phosphorylation → CheB methylesterase

Excitation and Adaptation are coupled

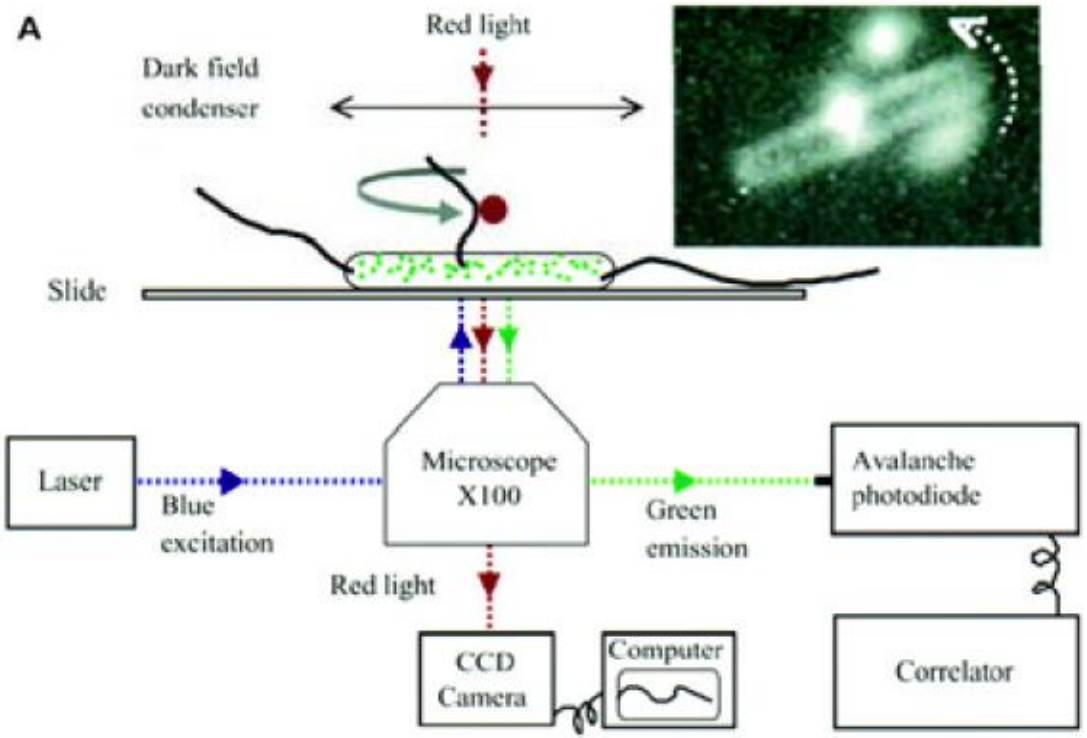
- Functions as a pulse generator
  - Temporary response due to faster kinetics eventually repressed by slower negative feedback system

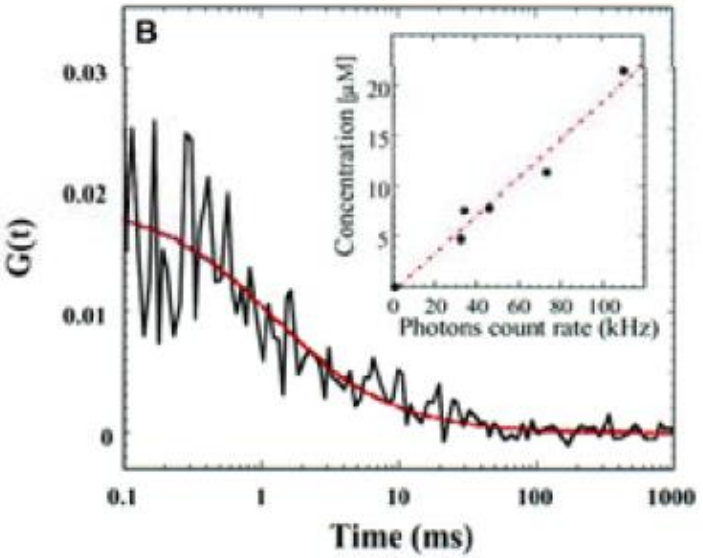


Before starting with the modeling,
first let's look at some recent experiments

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)

Experimental procedure to analyze flagella rotation directions



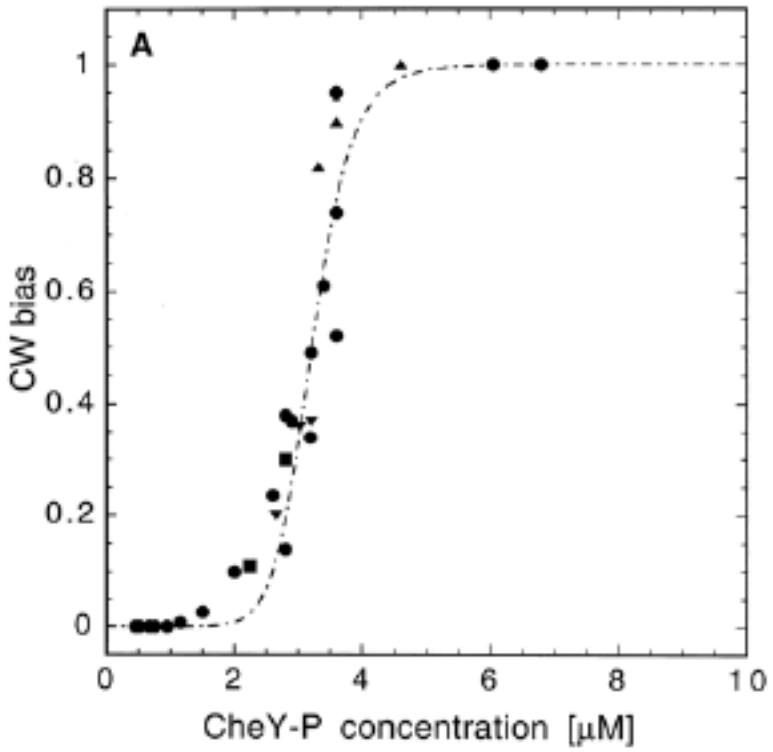


Single cell chemotactic analysis

Cluzel Science 2000

Flagella rotation is very sensitive to cheY phosphorylation

correlation CW bias & CheY-P gene expression



cells have plasmids with CheY-GFP under inducible promoter

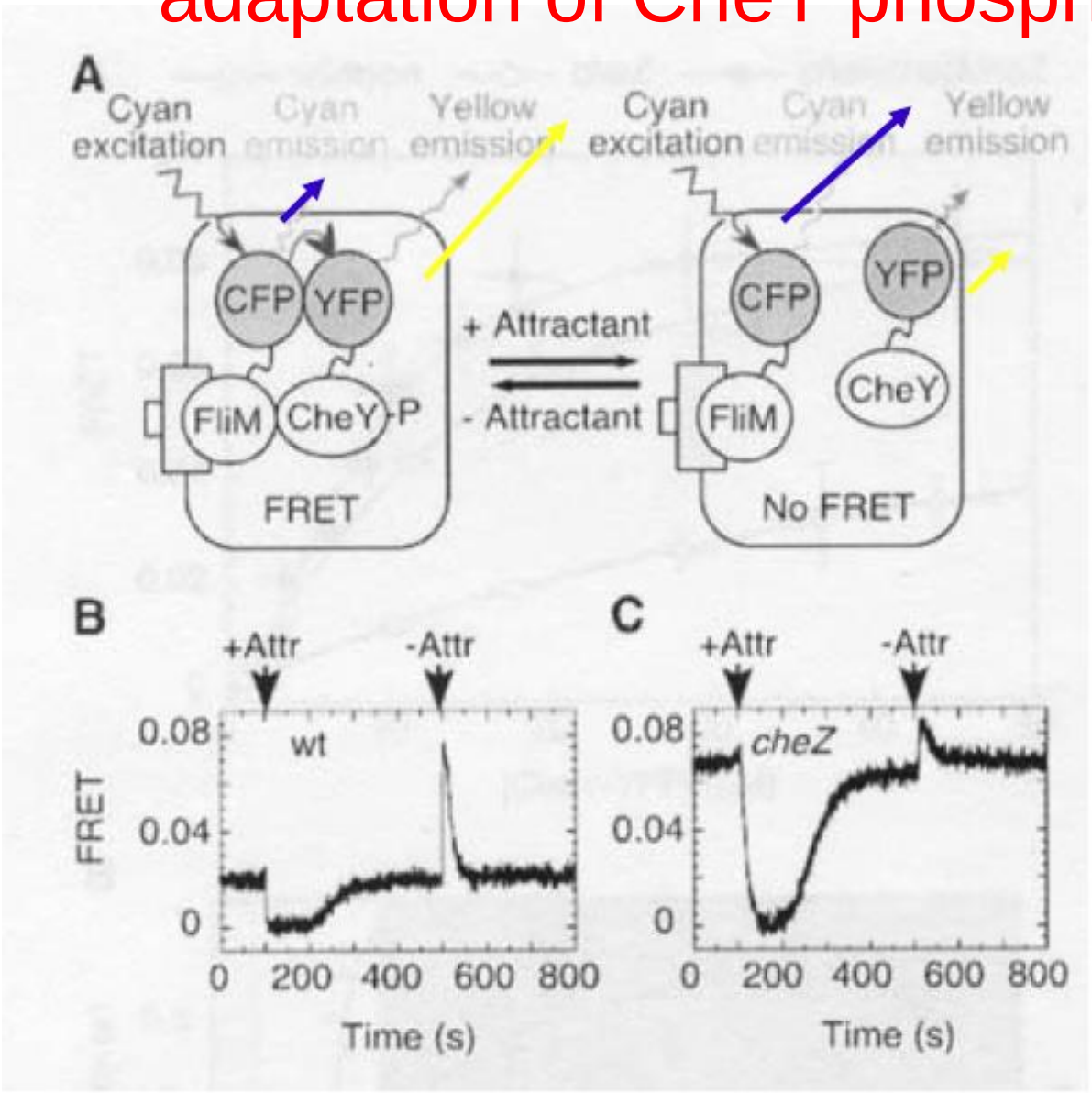
assumption: all CheY is phosphorylated

strain: CheY-, CheZ-, CheB-

Hill #: ~10

What about measurement of direct binding of CheY-p to motor?

FRET and cheYp-FliM interaction indicate adaptation of CheY phosphorylation



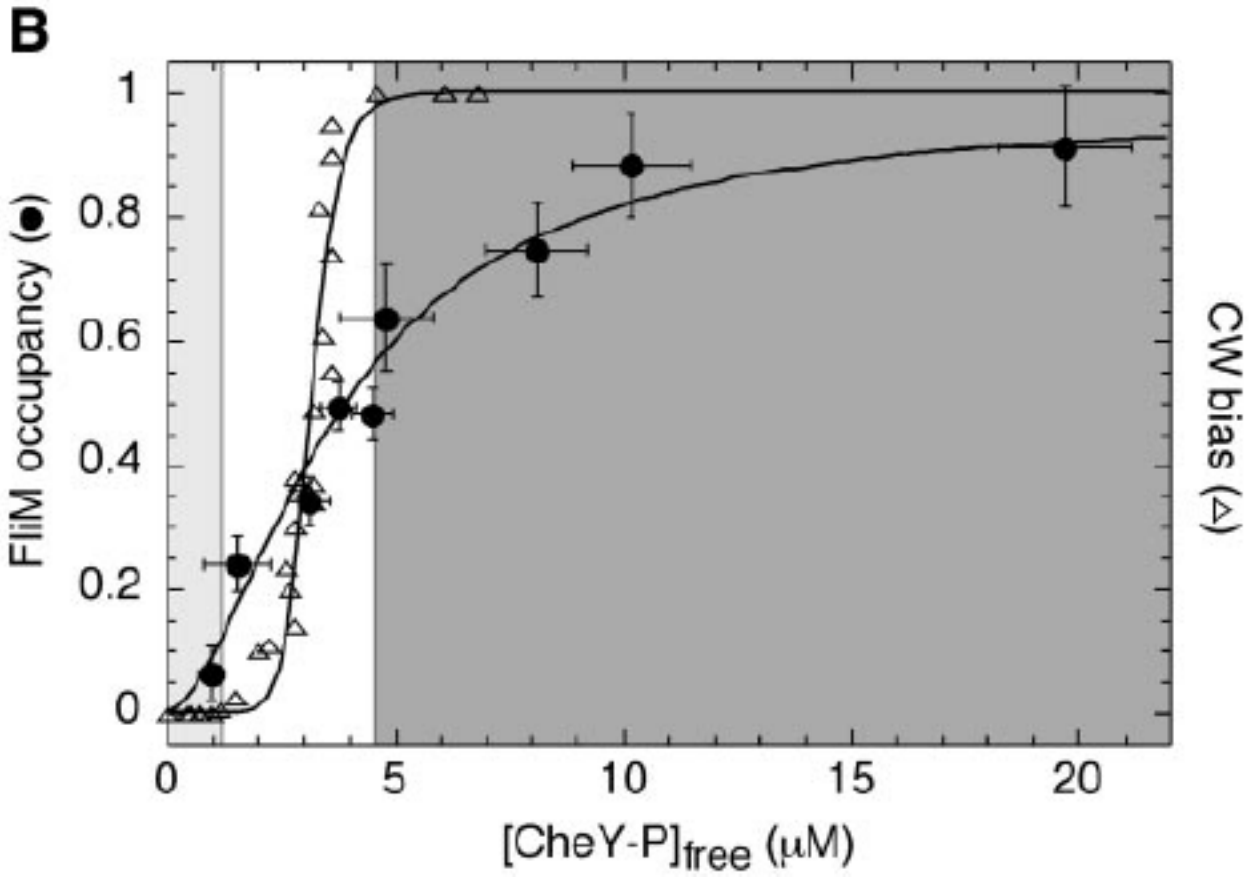
low YFP/CFP: unbound

high YFP/CFP: bound

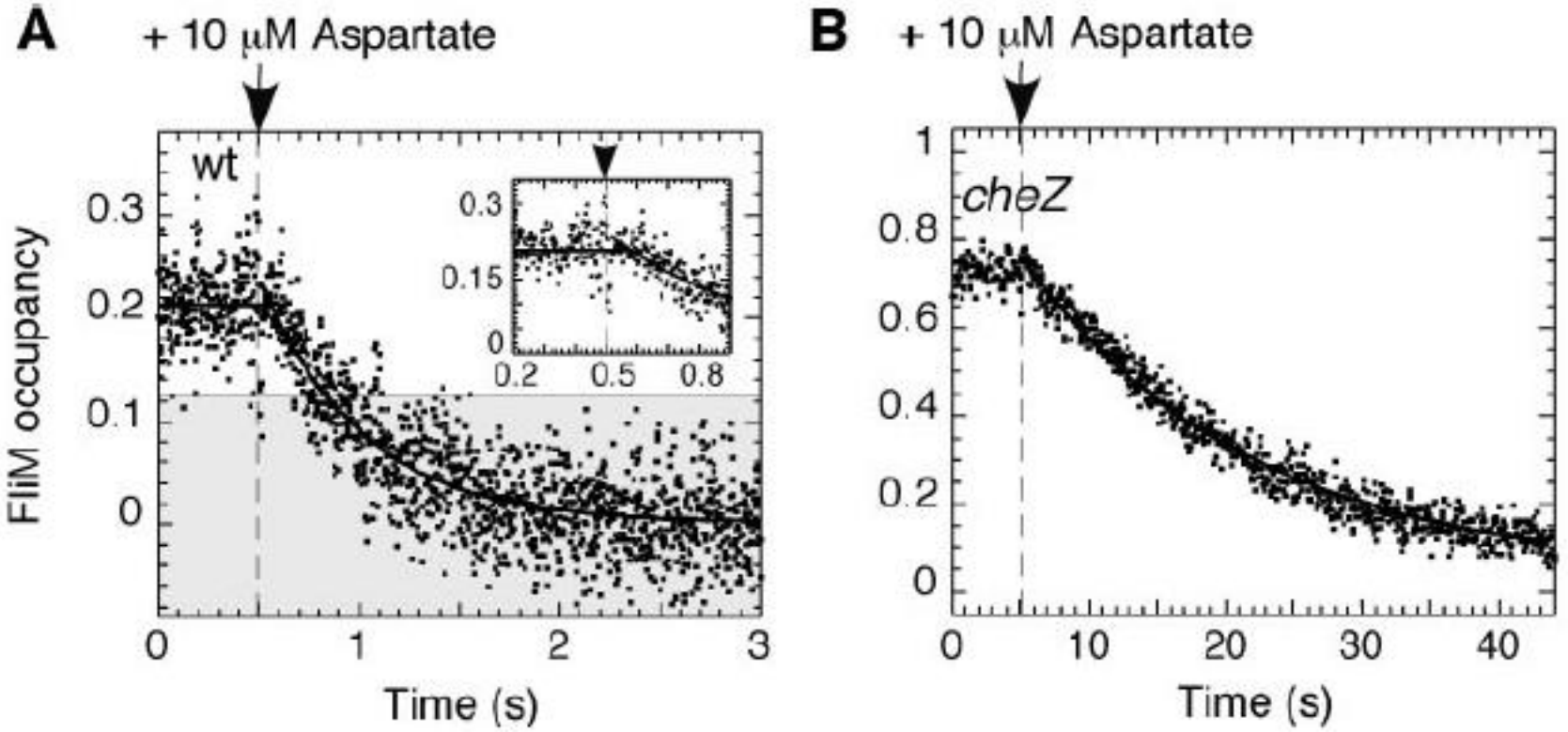
Sourjik PNAS 2000

FRET (fluorescence resonant transfer)

FliM-cheYp interaction is less cooperative than Motor output



cheZ speeds up the FliM response to Aspartate

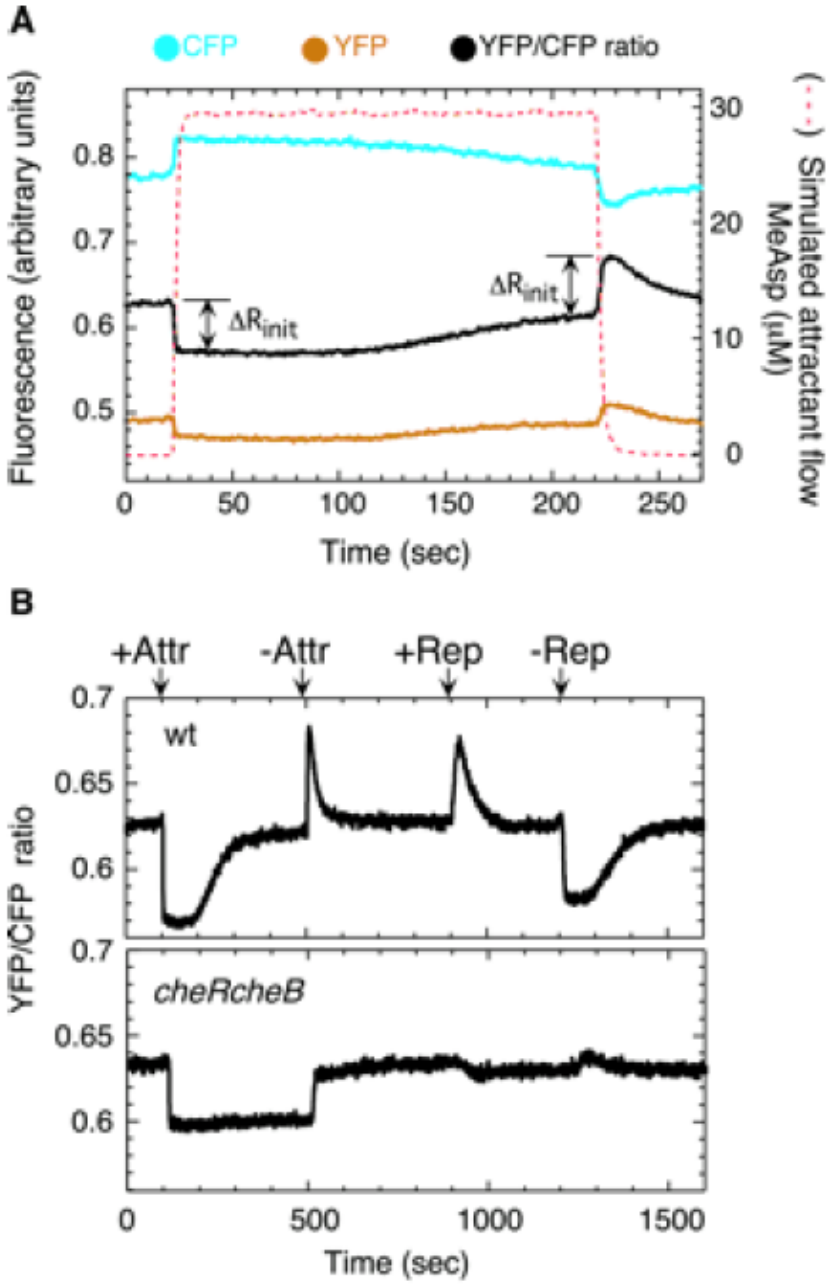


FRET-based adaptation of CheZ-CheYp interaction

CheY-YFP (yellow)
CheZ-CFP (blue

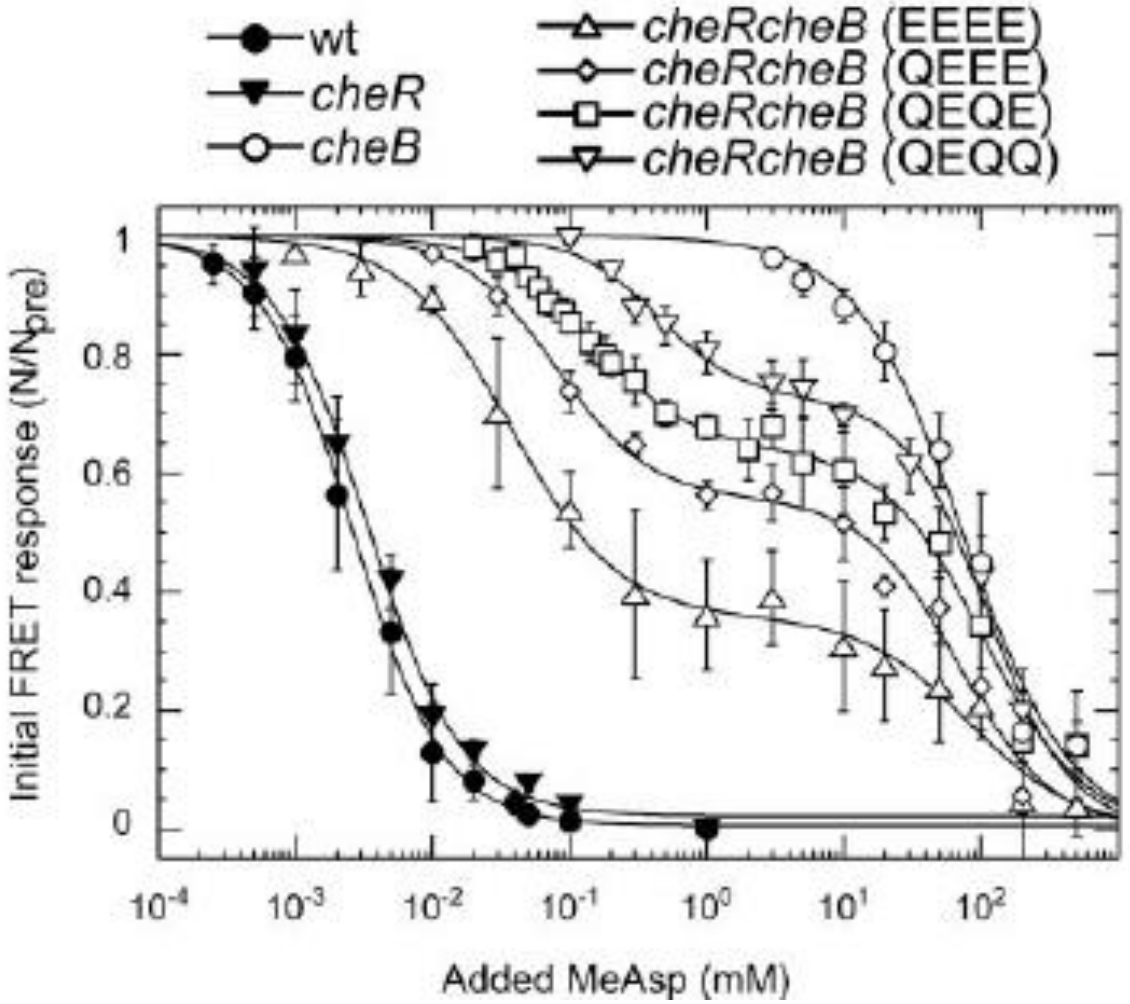
CheZ binds only to CheYp !!

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



Sourjik PNAS 2000

cheR cheB mutants show significantly wide range of sensitivities to Asp and no cooperativity



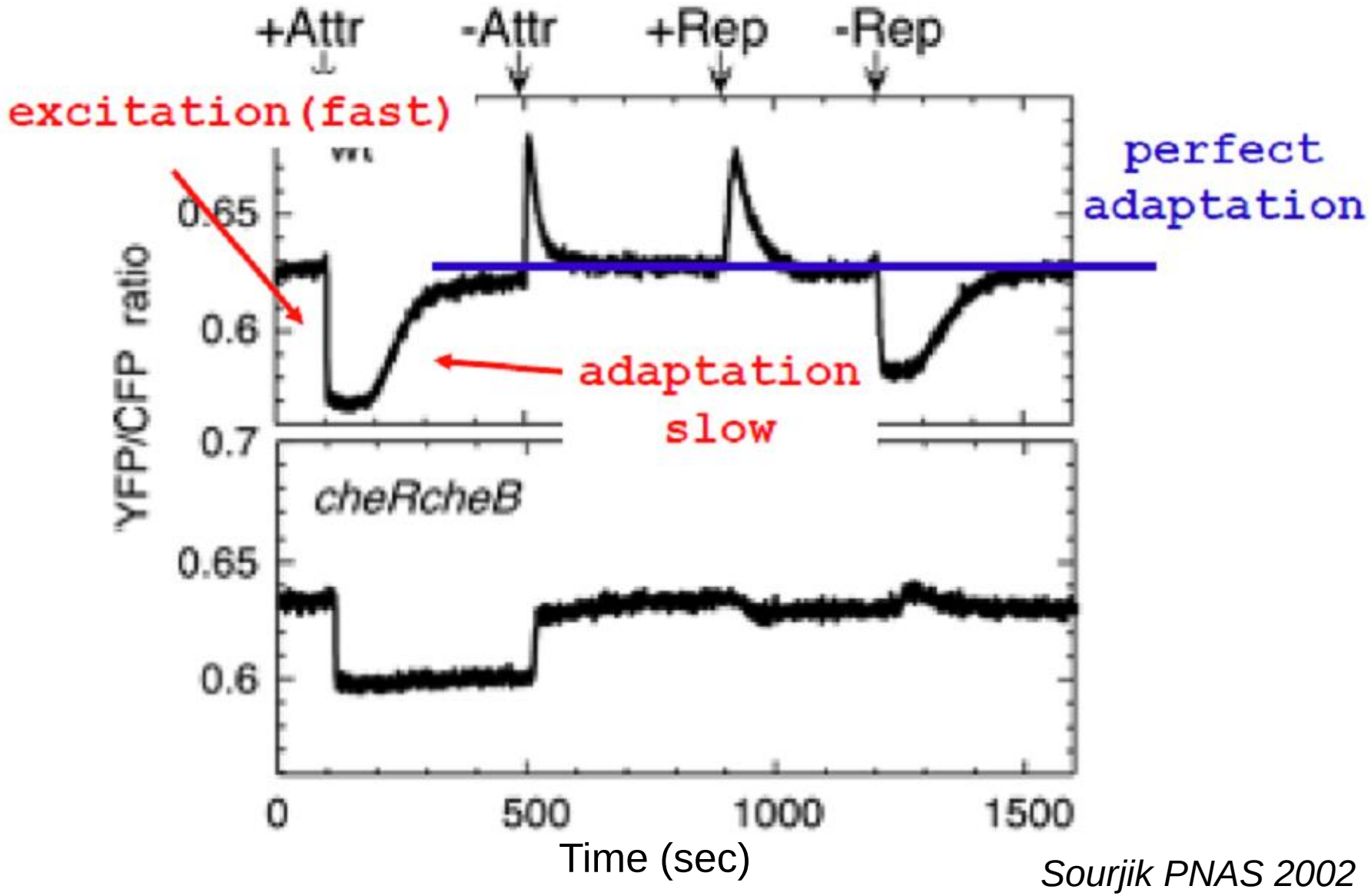
Sourjik PNAS 2002

Multistep reactions leads to high amplification

- Amplification between receptors and cheYp: ~35 folds
- Amplification between cheYp and motor: ~10 folds
- cheZ doesn’t cause amplification

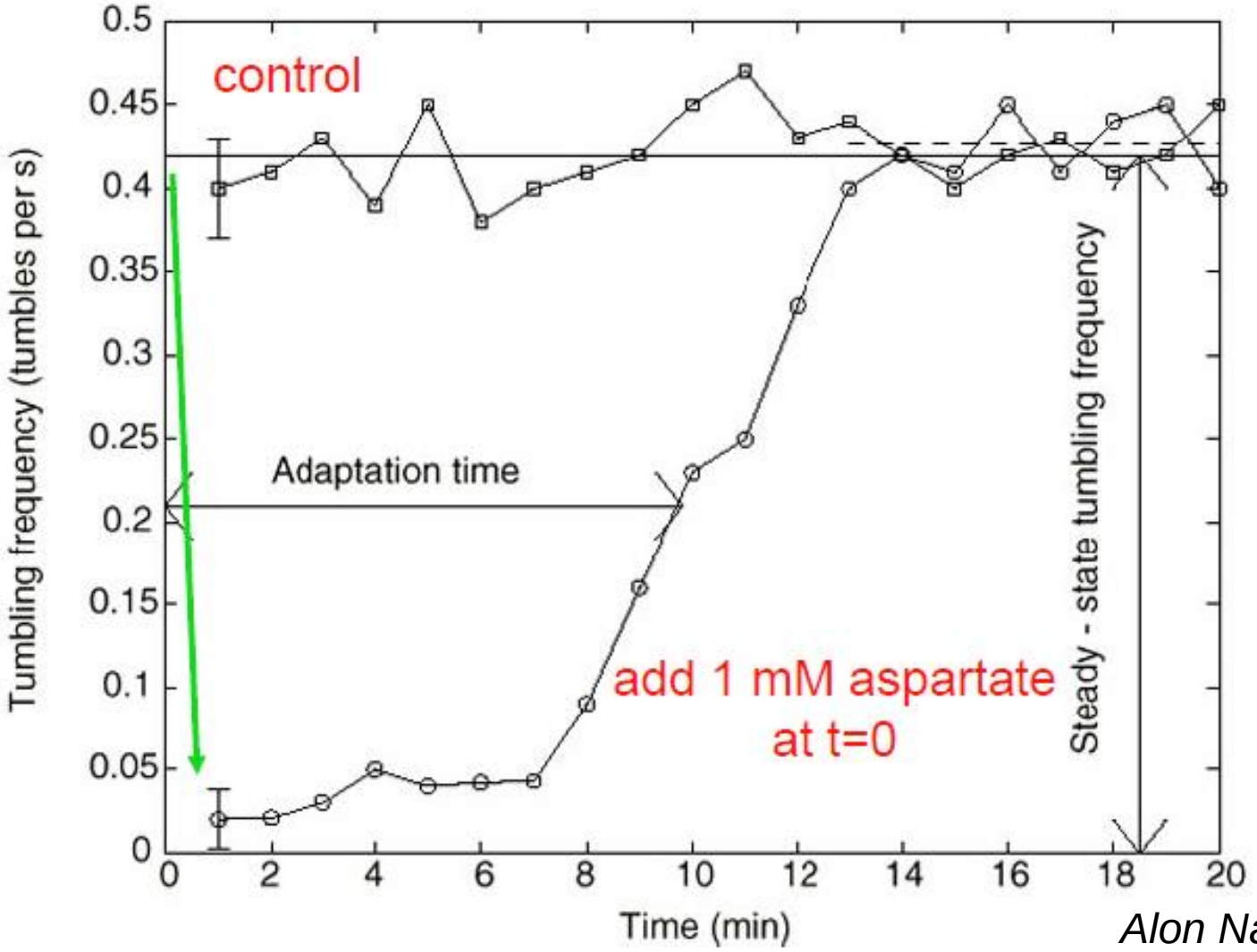
Sourjik PNAS 2002

Perfect adaptations



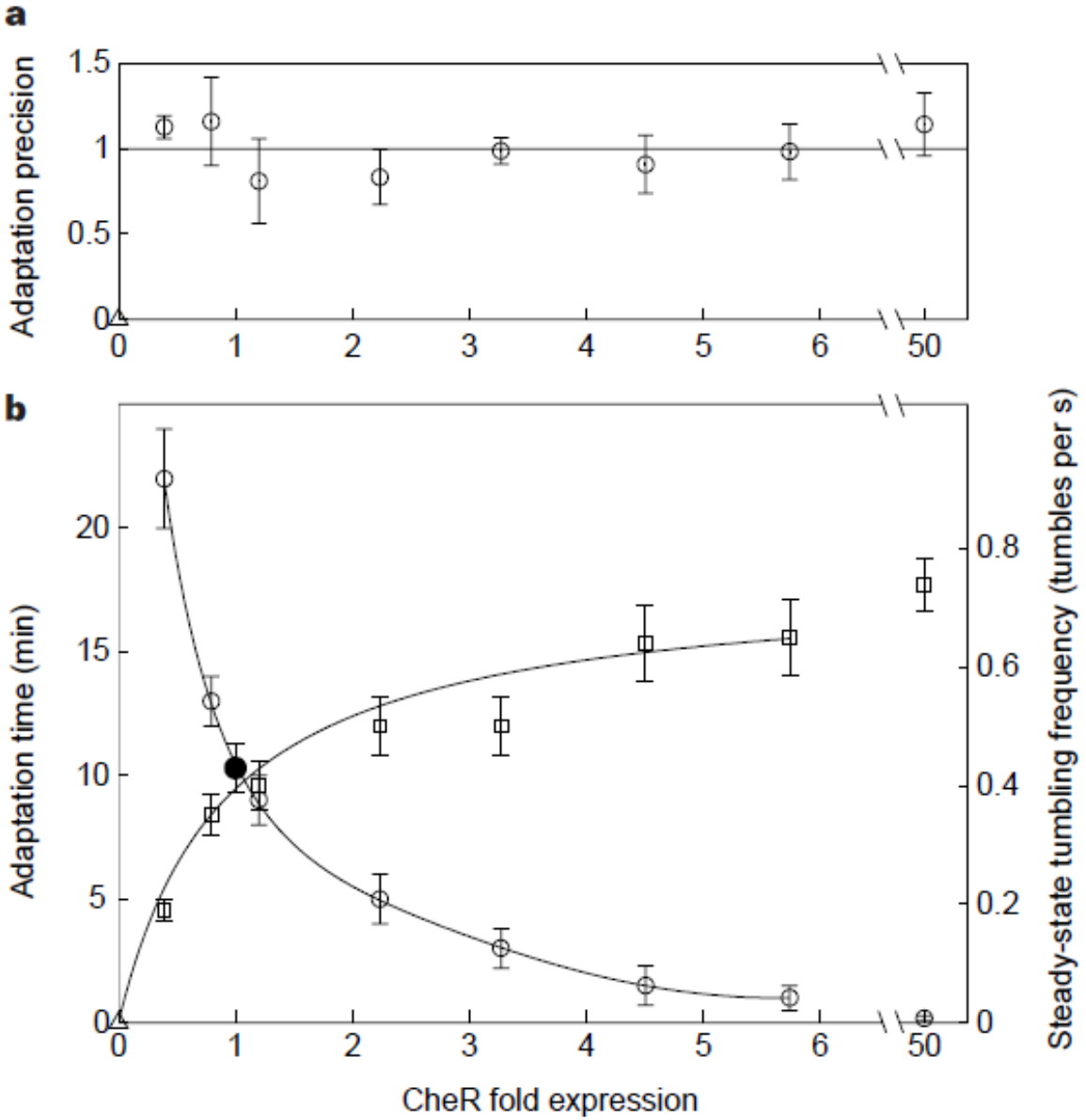
Sourjik PNAS 2002

The measurement of adaptation precision and time



Alon Nature 1999

Perfect adaptation is robust against changes in cheR expression but not adaptation time.



The goal of the model

- Huge gain
- Sensitivity
- Perfect adaptation

All these are ubiquitous in signal transduction networks in general.

What's the basis for perfect adaptation? Two explanations:

- The kinetic parameters are fine-tuned.
  - E. g.: Spiro et al. *A model of excitation and adaptation in bacterial chemotaxis*. *PNAS, 1997*

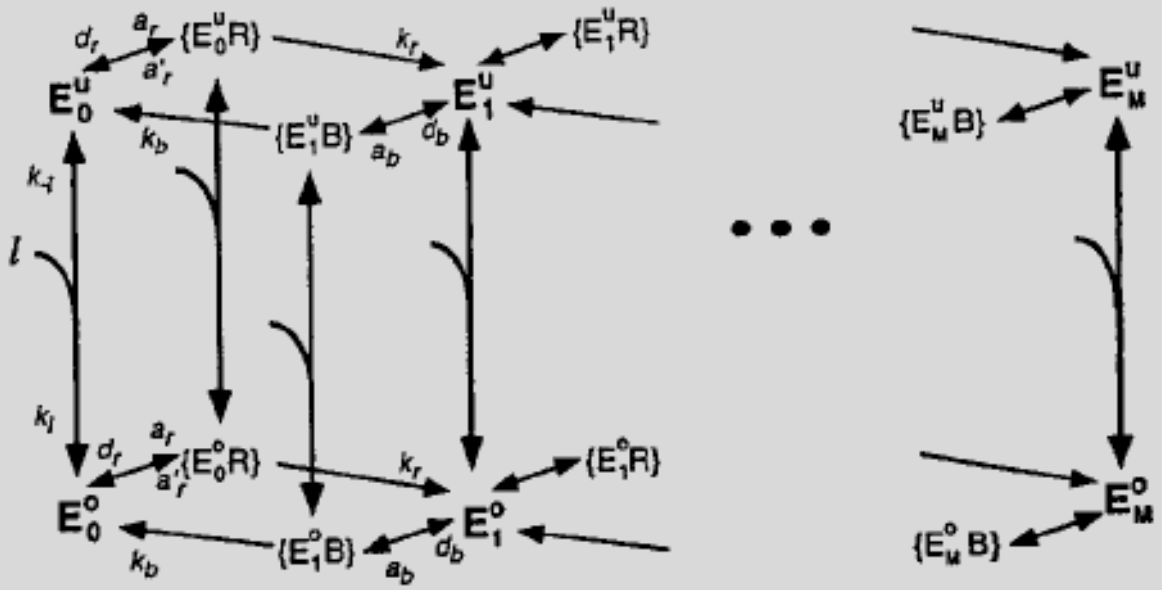
- Perfect adaptation is a robust property of the underlying network.
  - Barkai & Leibler 1997, Nature (Modeling)
  - Alon et al 1999, Nature (Experiment)

Assumptions in Barkai-Leibler model

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

Barkai&Leibler 1997 Naure 387, 913

A two-state model



The differential equations describing our model can be written in a standard way from the figure. For instance, the kinetic equation for E_m^u is

$$\frac{dE_m^u}{dt} = -k_l l E_m^u + k_{-l} E_m^0 +$$

$$\left(1-\delta_{m,0}\right)\left[-a_b \alpha_m E_m^u B + d_b\left\{E_m^u B\right\} + k_r\left\{E_{m-1}^u R\right\}\right] +$$

$$\left(1-\delta_{mM}\right)\left[-a_r \alpha_m E_m^u R + a'_r\left(1-\alpha_m\right)E_m^u R + d_b\left\{E_m^u R\right\} + k_b\left\{E_{m+1}^u B\right\}\right]$$

$$\left(m = 1, \ldots M\right)$$

Barkai&Leibler 1997 Naure 387, 913

Testing Robustness

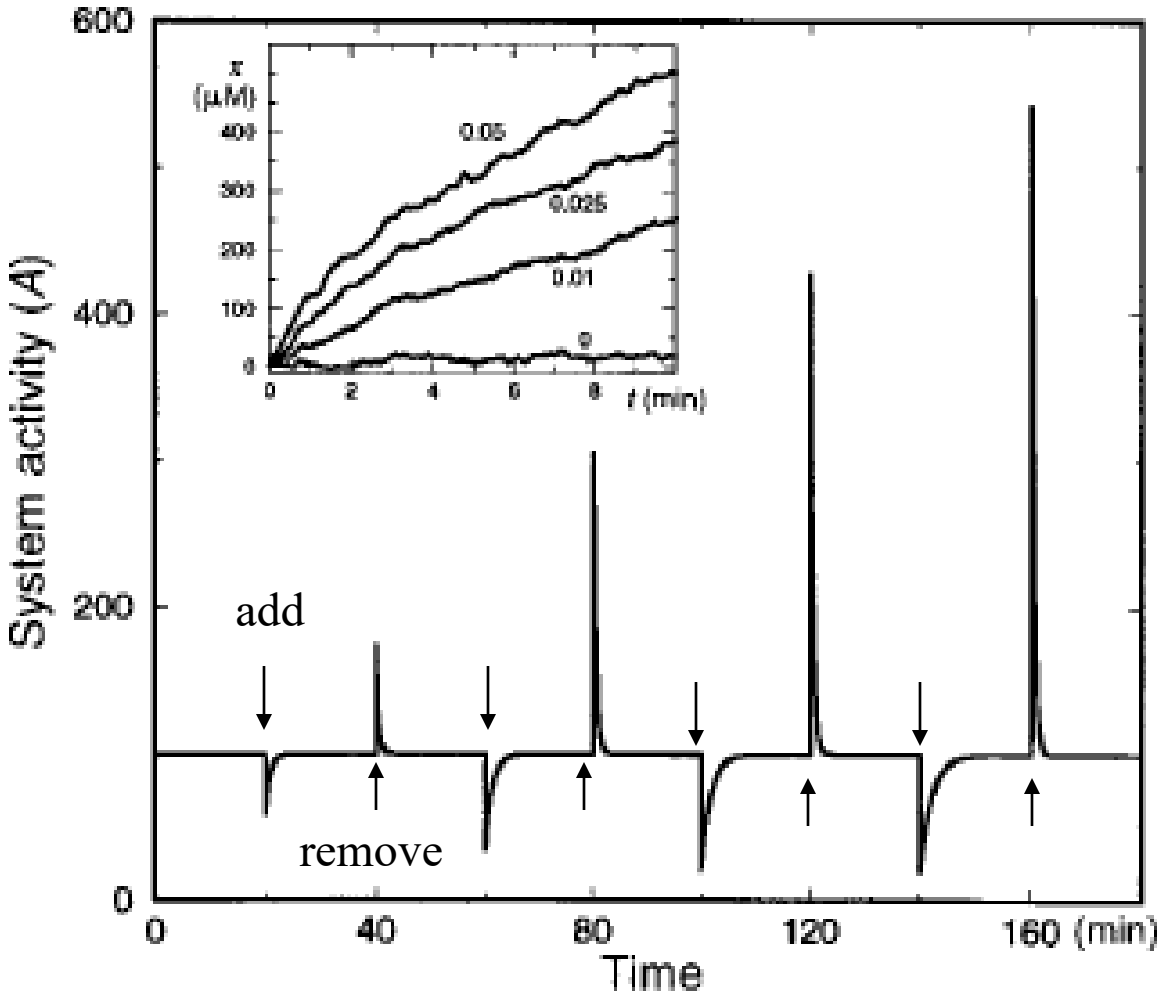
Model has 9 rate constants and 3 enzyme concentrations

In each simulation increase or decrease each parameter 2X randomly

Overall parameter Change = $P_{n,1}/P_{o,1}$ x $P_{n,2}/P_{o,2}$ x …….

Do 6000 independent simulations

Examine effects on precision and timing of adaptation



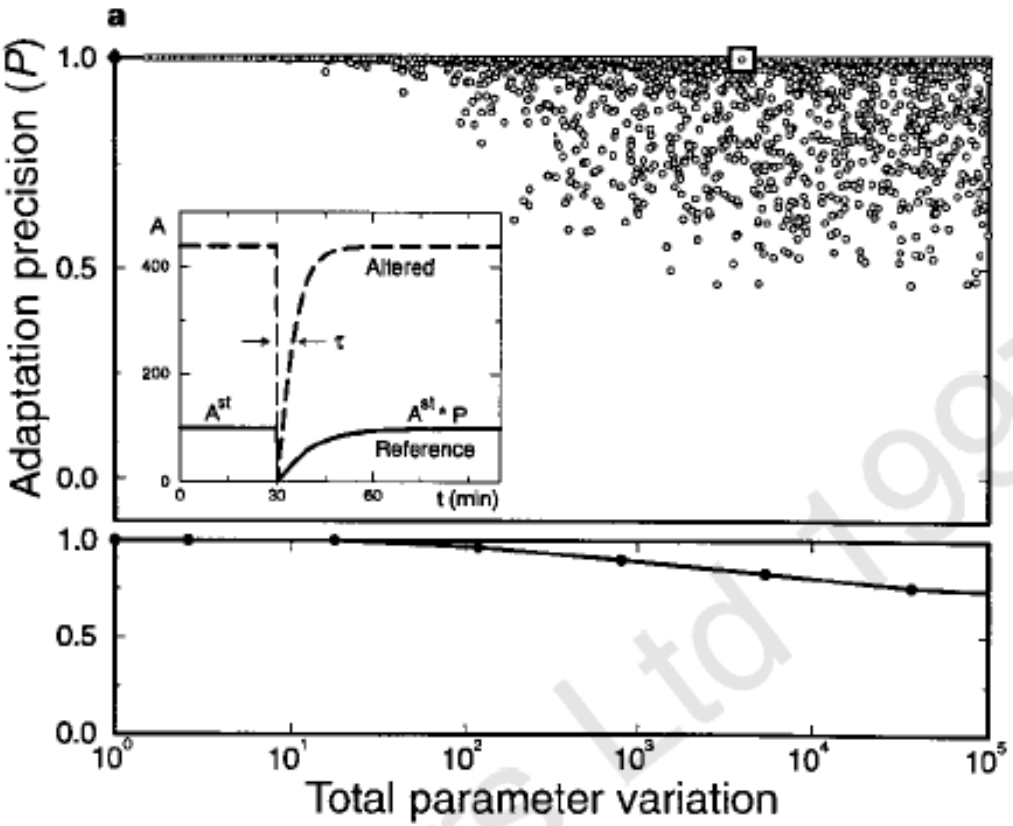
Modeling the effect of successive addition and removal (after 20 min.) of 1, 3, 5 and 7 mM of chemoattractant

Barkai&Leibler 1997 Naure 387, 913

All rate constants in the model can be changed randomly by 2 fold with only a ~15% deviation from perfect adaptation.

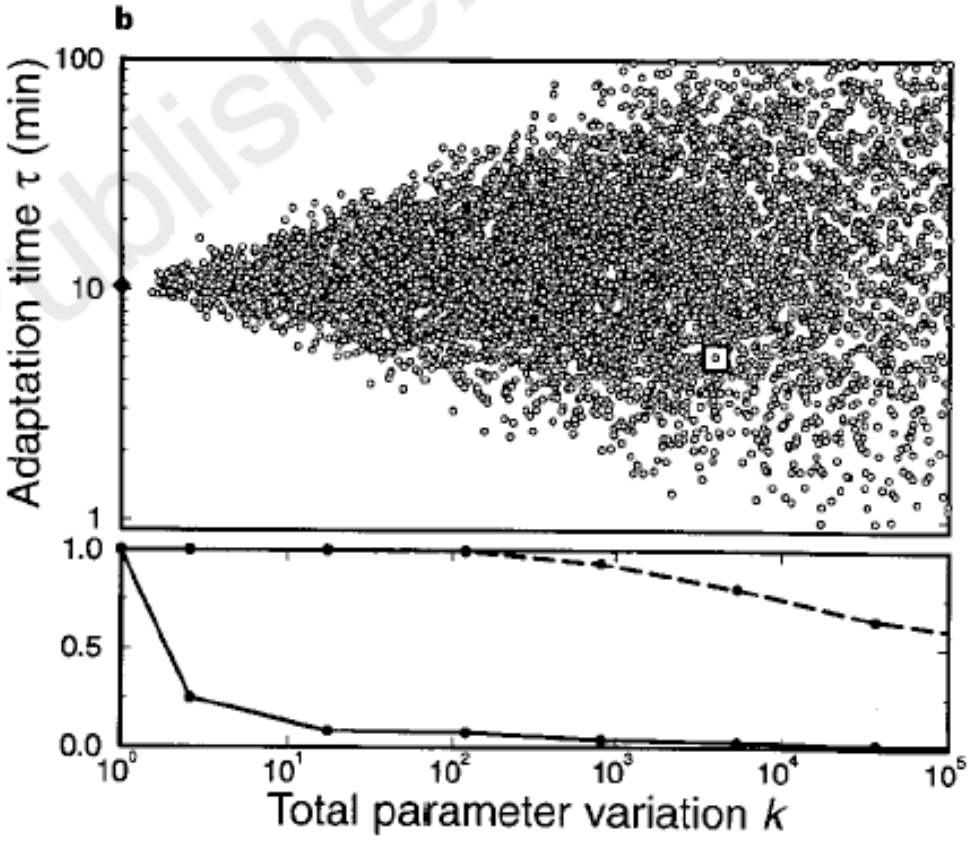
A single rate constant can be changed by several orders of magnitude (holding other parameters constant) without significant deviation from perfect adaptation.

Multiple methylation is not necessary for robustness but improves adaptation response time.



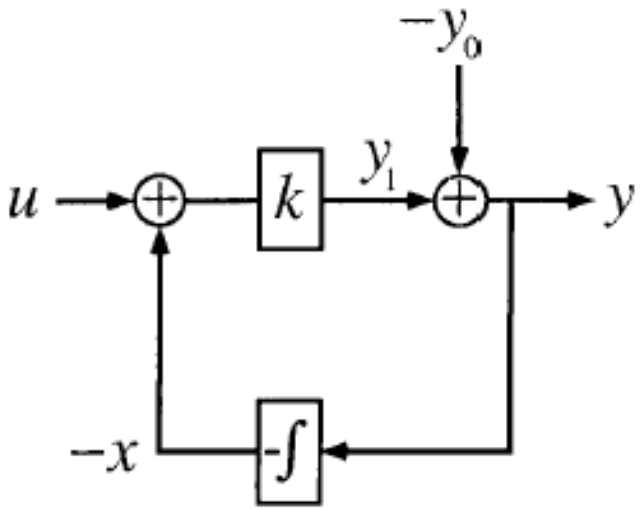
Barkai&Leibler 1997 Naure 387, 913

The time required for adaptation varies widely as the rate constants for the model are randomly changed.



Barkai-Leibler Model is a form of Integral Control

Yi, Huang, Simon&Doyle 2000 PNAS 97, 4649

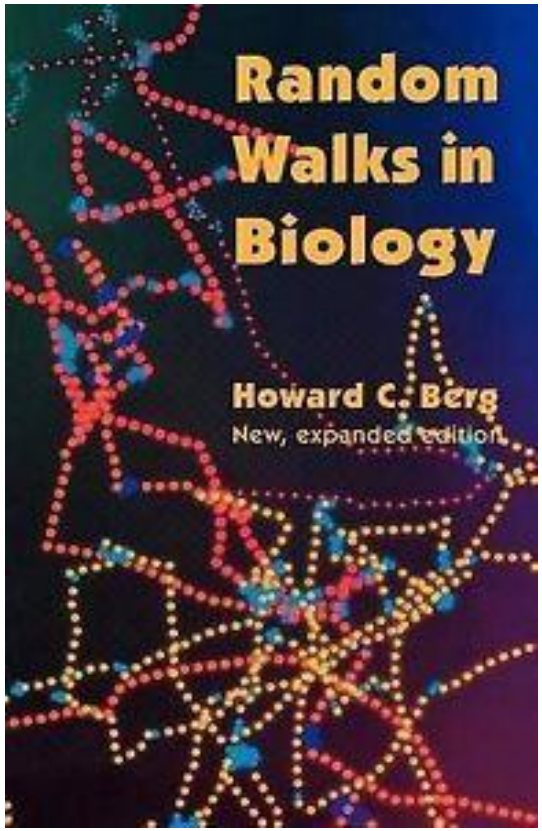


$\dot{x}=y$	$y(t)\to 0$ as $t\to\infty$
$y=y_1-y_0$	*iff*
$=k(u-x)-y_0$	$k>0$

Read the paper if you are interested.

Basic concepts of diffusion and chemotaxis

population vs individual



Diffusion

- Previously we assumed that the state of a cell at a particular point in time could be described by the concentrations of key components.
- The concentrations could change over time
- We implicitly assumed that within the cell these concentrations do not vary spatially inside the cell
- We considered the cell as a well-stirred biochemical reactor

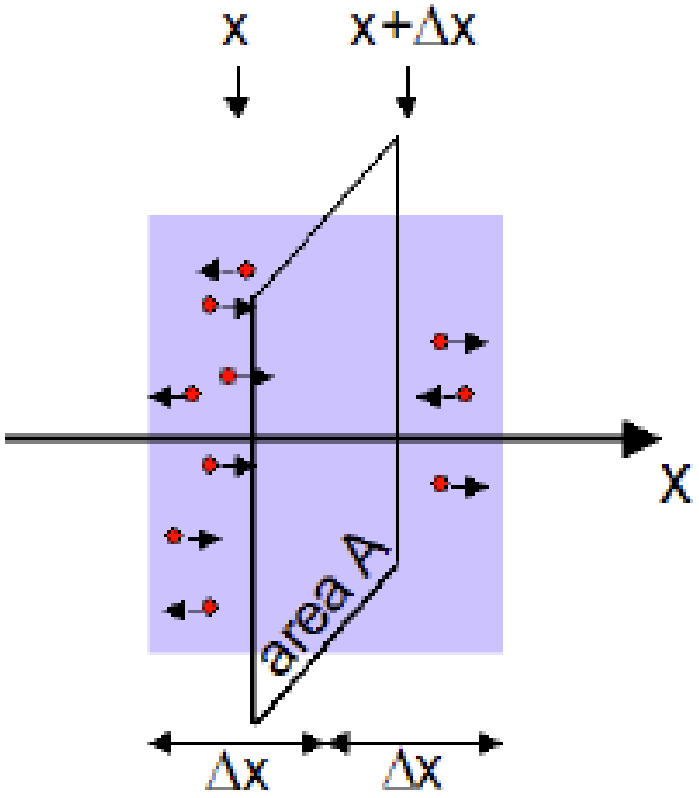
Where does the assumption fail

- For small cells such as E coli this is often a valid assumption
  - Dynamical phenomena:
    - time scale:seconds-minutes.
  - Random mixing by diffusion:
    - time scale ~L^2/D~0.1s, L~1μm, D~10μm^2/sec
- Larger cells such as eukaryotic cells
- Fast process in small cells
- Signal molecules diffusion between cells etc

Fick's first law

To understand the spatial inhomogeneous systems we take a look at Fick's laws

- Considering particles randomly moving along one-dimension crossing an area A.
- Assuming there are N(x) particles on the left of area A and N(X+ΔX) particles on the right of area A



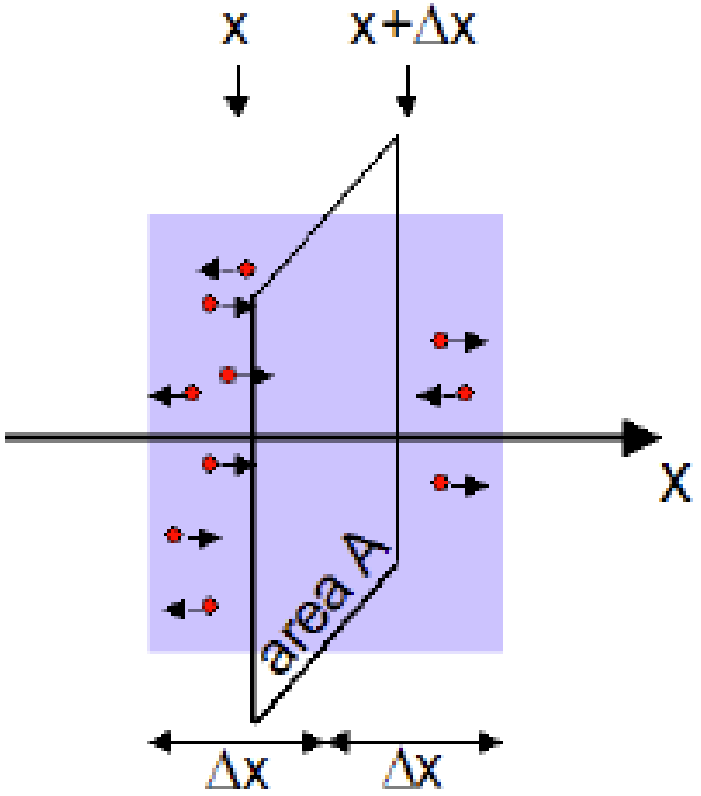
Fick's first law

Probability of traveling to left and right are identical. 0.5N(x) will travel to the right cross area A.

However, 0.5N(x+Δx) will travel to the left cross area A.

Therefore the net number of crossing to the right is:

$$-\frac{1}{2}\left(N(x+\Delta x)-N(x)\right).$$



Fick's first law

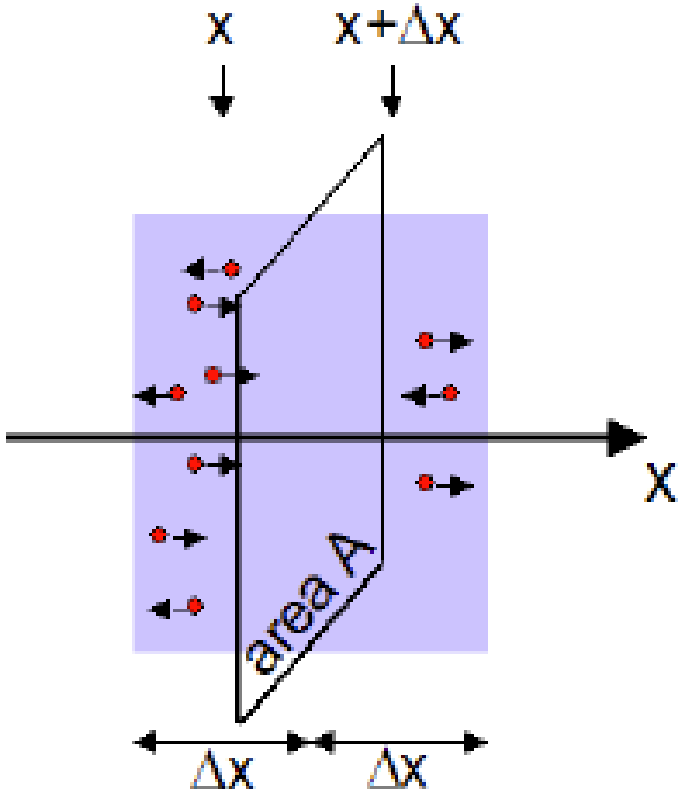
The flux of molecules J through the area A during a short time interval τ is defined as:

$$J = \frac{-\frac{1}{2}\left(N(x+\Delta x) - N(x)\right)}{A\tau}$$

Defining concentration results in:

$$C(x) \equiv \frac{N(x)}{A\Delta x}$$

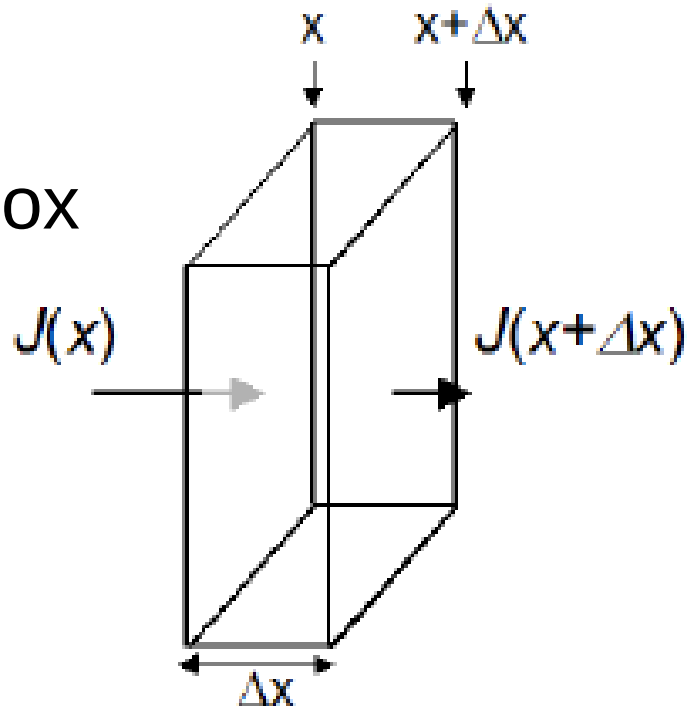
$$J = -\frac{(\Delta x)^2}{2\tau}\frac{\left(C(x+\Delta x) - C(x)\right)}{\Delta x} = -D\frac{\partial C(x)}{\partial x}$$



Where D is defined as the diffusion constant.

Fick's second law

Assuming a particle cannot be created or destroyed, consider the volume AΔx, A flux J(x) enter the box from the left and a flux J(x+Δx) leaves the box to the right.



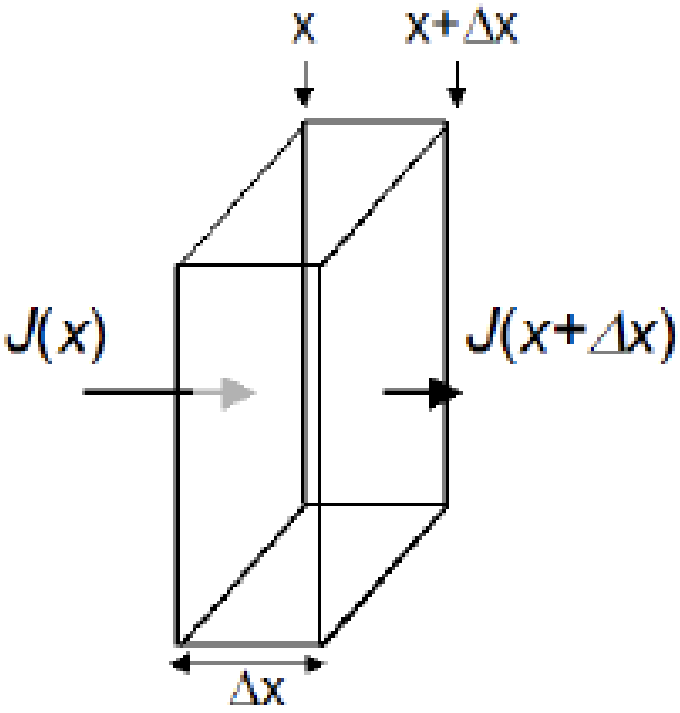
The rate of change of the concentration in the box during a short time τ is given by:

$$\frac{C(t+\tau)-C(t)}{\tau}=\frac{1}{\tau}\frac{\left(J(x)-J(x+\Delta x)\right)A\tau}{A\Delta x}=-\frac{\left(J(x+\Delta x)-J(x)\right)}{\Delta x}$$

Fick's second law

- Taking the limit $t \rightarrow 0$ and $\Delta x \rightarrow 0$ results in:

$$\frac{\partial C(x)}{\partial t} = -\frac{\partial J(x)}{\partial x}$$



- Combining it with the first law lead to the second law

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2}$$

Random walks

Let us consider a one-dimensional random walk of N particles. A particle always takes a fixed step size Δx toward the left or right with equal probability.

The position of the particle is denoted by $X_j(n)$, where the subscript denotes which particles and n denotes the number of steps that particle j took.

All particles starts their random walk at x=0. The position average of all particles remains zero:

$$\langle x(n) \rangle = \sum_{i=1}^{N} x_i(n) = \sum_{i=1}^{N} \left(x_i(n-1) \pm \Delta x \right) = \sum_{i=1}^{N} x_i(n-1) = \sum_{i=1}^{N} x_i(0) = 0$$

Random walks

However individual particles are spreading in both the positive and negative direction,

A convenient way to quantify the spreading is to calculate the variance of the distribution of positions. The variance is defined as:

$$Var(x(n)) = \langle x^2(n) \rangle - \langle x(n) \rangle^2$$

$$Var(x(n)) = \langle x^2(n) \rangle - 0 = \sum_{i=1}^{N} x_i^2(n) = \sum_{i=1}^{N} \left(x_i(n-1) \pm \Delta x \right)^2 = \langle x^2(n-1) \rangle + \Delta x^2$$

The variance is non-zero, and it grows by ΔX^2 for each steps, and since the variance is non-zero

$$\langle x^2(n) \rangle = n \Delta x^2$$

Random walks

During one step the particle moves at a constant velocity $v=\Delta x/\tau$. This means that a time t the particles performed $n=t/\tau$ steps, therefore the variance is proportional to t:

$$\left\langle x^2(n)\right\rangle = t\frac{\Delta x^2}{\tau} \equiv 2Dt$$

Random walk under external force

- Single particles: Langevin equations

$$m\frac{d^2x}{dt^2}+\gamma\frac{dx}{dt}=f(x)+\Gamma(t)$$

Inertia term

Damping term

deterministic force

random force

Random walk of biological cell

Damped Langevin equations

$$\gamma \frac{dx}{dt} = f(x) + \Gamma(t)$$

$$\Gamma(t) = \sqrt{D / dt}\; g(t)$$

Damping term

deterministic force

random force

f(x), gravity, chemotaxis etc

A good approximation?

From damped Langevin equation of single cell/molecule, mathemacians derived:

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2}{\partial x^2}C(x,t) - \frac{\partial}{\partial x}[f(\ldots)C(x,t)]$$

In physics and mathematics, this type od equations is called Fokker-Planck Equations.

In finance, the famous Black-Scholes-Merton model for pricing of options is a derivation of this equations. Scholes and Merton won the Nobel prize in Economy in 1997 .

The Sveriges Riksbank Prize in Economic Sciences in Memory of Alfred Nobel 1997
Robert C. Merton, Myron Scholes

Share this:

The Sveriges Riksbank Prize in Economic Sciences in Memory of Alfred Nobel 1997

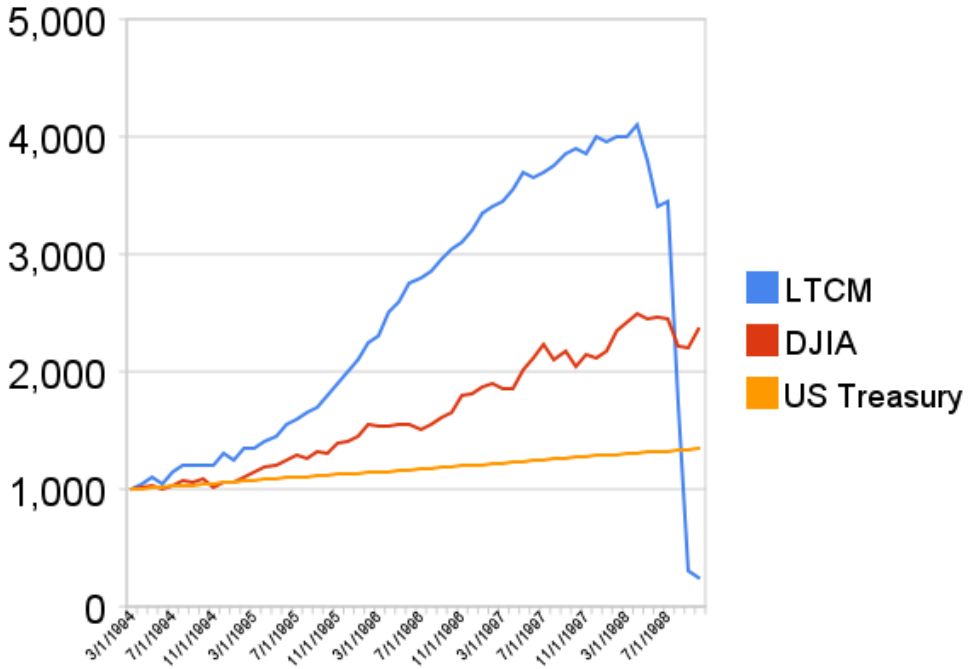
Robert C. Merton
Prize share: 1/2

Myron S. Scholes
Prize share: 1/2

The Sveriges Riksbank Prize in Economic Sciences in Memory o
Alfred Nobel 1997 was awarded jointly to Robert C. Merton an
Myron S. Scholes *"for a new method to determine the value of
derivatives"*

Myron S. Scholes (left) and Robert C. Merton were principals at LTCM.

Long Term Capital Management 1994-1998



1998 bailout [edit]

Long-Term Capital Management did business with nearly everyone important on Wall Street. Indeed, much of LTCM's capital was composed of funds from the same financial professionals with whom it traded. As LTCM teetered, Wall Street feared that Long-Term's failure could cause a chain reaction in numerous markets, causing catastrophic losses throughout the financial system.

After LTCM failed to raise more money on its own, it became clear it was running out of options. On September 23, 1998, Goldman Sachs, AIG, and Berkshire Hathaway offered then to buy out the fund's partners for $250 million, to inject $3.75 billion and to operate LTCM within Goldman's own trading division. The offer was stunningly low to LTCM's partners because at the start of the year their firm had been worth $4.7 billion. Warren Buffett gave Meriwether less than one hour to accept the deal; the time lapsed before a deal could be worked out.[24]

Seeing no options left, the Federal Reserve Bank of New York organized a bailout of $3.625 billion by the major creditors to avoid a wider collapse in the financial markets.[25] The principal negotiator for LTCM was general counsel James G. Rickards.[26] The contributions from the various institutions were as follows:[27][28]

- $300 million: Bankers Trust, Barclays, Chase, Credit Suisse First Boston, Deutsche Bank, Goldman Sachs, Merrill Lynch, J.P.Morgan, Morgan Stanley, Salomon Smith Barney, UBS
- $125 million: Société Générale
- $100 million: Paribas, Credit Agricole[29]
- Bear Stearns and Lehman Brothers[29] declined to participate.

On September 23, 1998, the chiefs of some of the largest investment firms of Wall Street —Bankers Trust, Bear Stearns, Chase Manhattan, Goldman Sachs, J.P. Morgan, Lehman Brothers, Merrill Lynch, Morgan Stanley Dean Witter, and Salomon Smith Barney—met on the 10th floor conference room of the Federal Reserve Bank of New York (*pictured*) to rescue LTCM.

In return, the participating banks got a 90% share in the fund and a promise that a supervisory board would be established. LTCM's partners received a 10% stake, still worth about $400 million, but this money was completely consumed by their debts. The partners once had $1.9 billion of their own money invested in LTCM, all of which was wiped out.[30]

The fear was that there would be a chain reaction as the company liquidated its securities to cover its debt, leading to a drop in prices, which would force other companies to liquidate their own debt creating a vicious cycle.

The total losses were found to be $4.6 billion. The losses in the major investment categories were (ordered by magnitude):[20]

- $1.6 bn in swaps
- $1.3 bn in equity volatility
- $430 mn in Russia and other emerging markets
- $371 mn in directional trades in developed countries
- $286 mn in Dual-listed company pairs (such as VW, Shell)
- $215 mn in yield curve arbitrage
- $203 mn in S&P 500 stocks
- $100 mn in junk bond arbitrage
- no substantial losses in merger arbitrage

Diffusion coefficients of proteins in solution and in cell

TABLE 1. Diffusion coefficients determined for GFP constructs in the cytoplasm of *E. coli* cells (unless indicated otherwise)[a]

Protein[b]	Molecular mass (kDa)	D ($\mu m^2 s^{-1}$)	Treatment	Source or reference
GFP in water	27	87		26
GFP	27	7.7 ± 2.5	Induced with 100 μM IPTG[c]	6
GFP	27	3.6 ± 0.7	Induced with 1 mM IPTG	6
EYFP	26.5	7.08 ± 0.3		12
GFP-His_6	27+	4.0 ± 2.0		6
cMBP-GFP	72	2.5 ± 0.6		6
CheY-GFP	41	4.6 ± 0.8		3
CFP-CheW-YFP	71	1.5 ± 0.05		12
CFP-CheR-YFP	86.2	1.7 ± 0.05		12
torA-GFP	30	9.0 ± 2.1	Cephalexin	18
GFP	27	9.8 ± 3.6	Cephalexin	28
GFP	27	0.4 ± 0.3	After osmotic upshock with NaCl	28
GFP	27	6.3 ± 1.1		25
GFP	27	3.1 ± 1.0	After osmotic shock	25
torA-GFP2 in Δ*tatABCDE* strain	57	7.5 ± 3.9	Cephalexin, 2% arabinose	This study
GFP2	27	9.1 ± 5.1	Cephalexin	This study
torA-GFP2	57	8.3 ± 4.2	Cephalexin, 500 μM arabinose	This study
torA-GFP3	84	6.3 ± 2.6	Cephalexin, 200 μM arabinose	This study
torA-GFP4	111	5.5 ± 1.9	Cephalexin, 1 mM arabinose	This study
torA-GFP5	138	2.8 ± 1.5	Cephalexin, 800 μM arabinose	This study
AmiA-GFP	58	1.8 ± 0.8	2% arabinose	This study
AmiA-GFP	58	1.8 ± 1.2	Cephalexin, 2% arabinose	This study
$AmiA_{noSP}$-GFP	58	7.1 ± 3.6	Cephalexin, 2% arabinose	This study
NlpA-GFP	55	2.1 ± 1.4	Cephalexin, 2% arabinose	This study
$NlpA_{noLB}$-GFP	55	2.7 ± 3.2	Cephalexin, 2% arabinose	This study

[a] The techniques used were FRAP and photoactivation of a red-emitting fluorescence state of GFP (6), fluorescence correlation spectroscopy (3), confocal FRAP (12, 18), pulsed FRAP (28), and continuous photobleaching with evanescent illumination (25).
[b] EYFP, enhanced yellow fluorescent protein; cMBP, cytoplasmic maltose-binding protein.
[c] IPTG, isopropyl-β-D-thiogalactopyranoside.