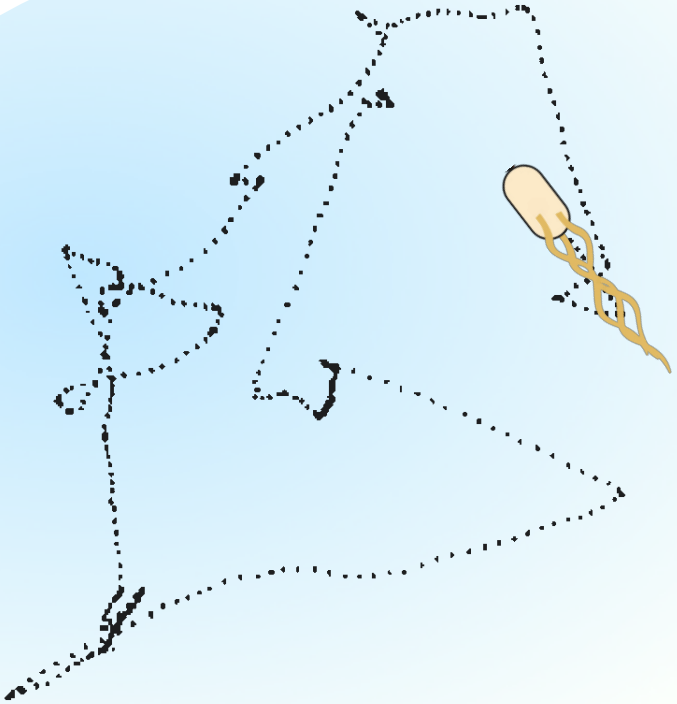
Cellular Motility



Course 5: Chemotaxis 1 – Bacteria

Thomas Lecuit

chaire: Dynamiques du vivant

COLLÈGE DE FRANCE 1530

Guidance of motility by the environment

Fonction

- source of energy/nutrition:

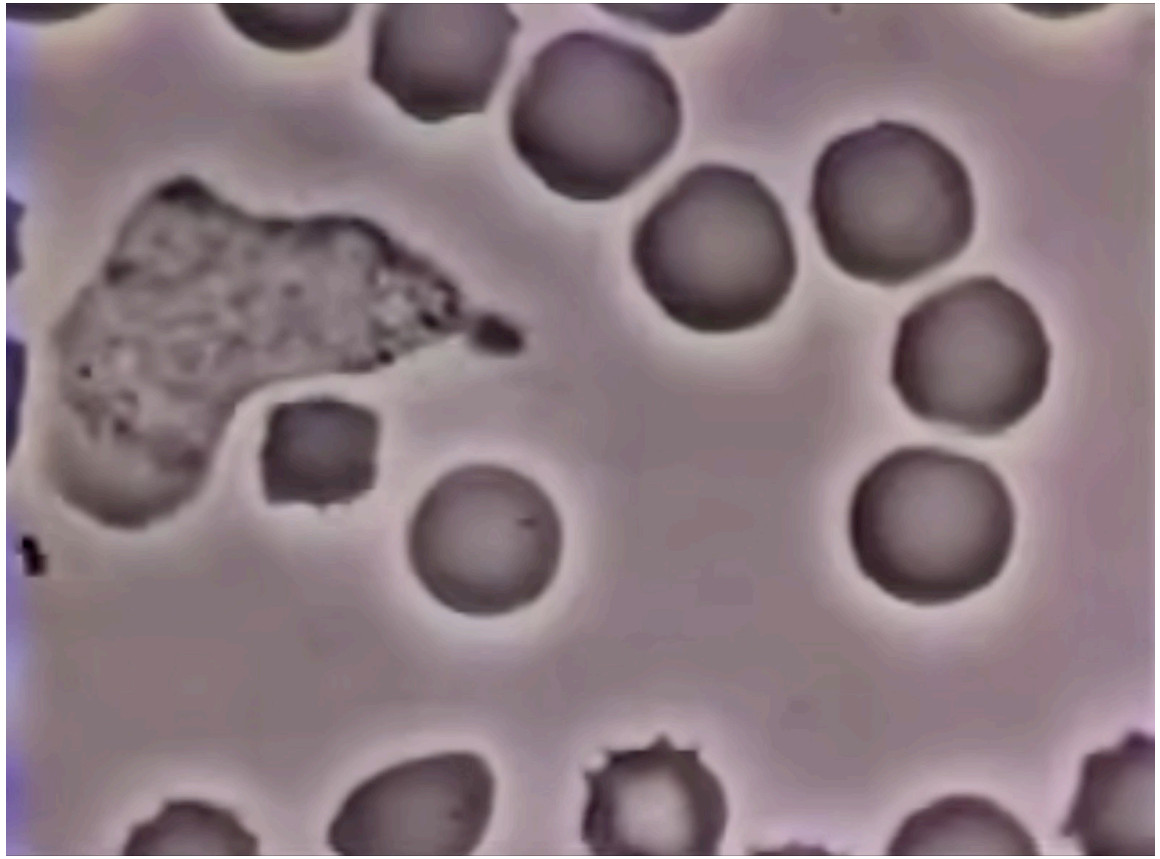
phototaxis (eg. *Volvox, Euglena*), glucose (*E. coli*), anaerobic conditions (e.g. magnetotactic bacteria)

- reproduction: sperm cells
- escape from predators/toxins
- patrolling: immune defense (eg. dendritic cells)
- embryonic development
- regeneration-repair

PLoS ONE 11(10): e0162602. doi:10.1371/journal.pone.0162602

Guidance of cell motility

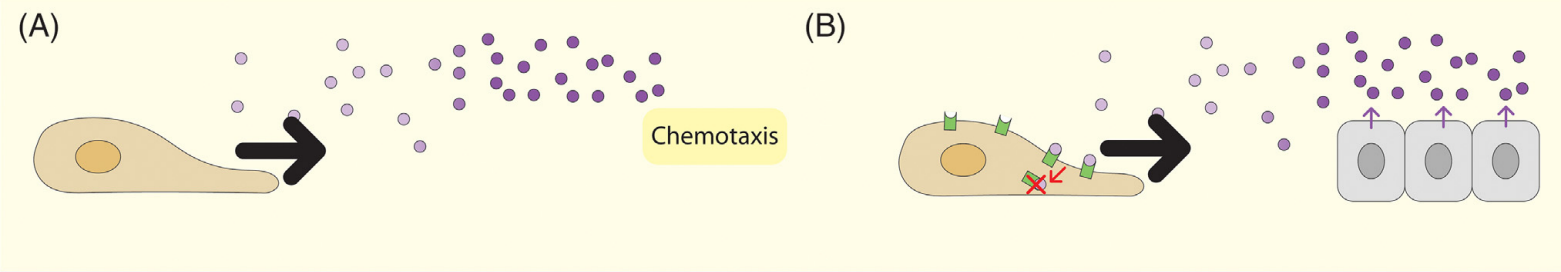
- Neutrophile chasing a bacterium (*Staphilococcus aureus*)

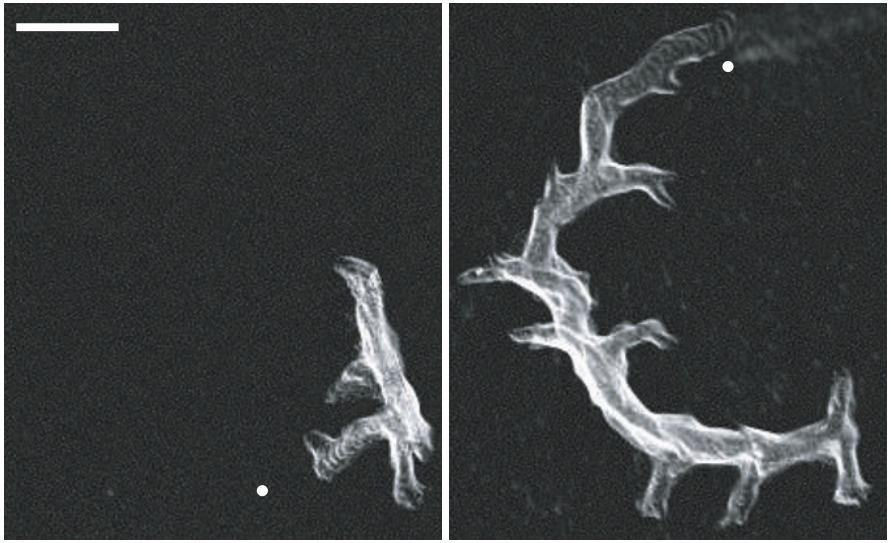


David Rogers at Vanderbilt University. https://www.youtube.com/watch?v=I_xh-bkiv_c

Nature of guidance cues

– Chemical cues: Chemotaxis





Dictyostelium discoideum

Migration mode	Cue	Signal generation
Chemotaxis	Diffusible chemical released from cells or deposited extracellular vesicles	Simple diffusion Regulated removal by degradation of the chemoattractant or decoy receptors Release of extracellular vesicles

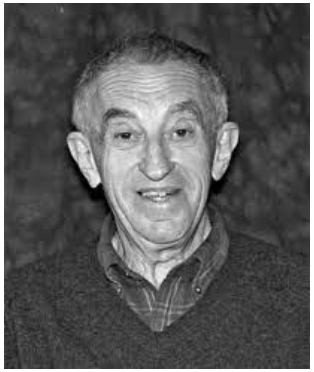
S. SenGupta, C. A. Parent and J. E. Bear, *Nature Rev Mol. Cell Biol.* 2021 https://doi.org/10.1038/ s41580-021-00366-6

Chemical guidance – Chemotaxis

1. Bacterial chemotaxis

2. Eukaryote chemotaxis

Chemical guidance of bacteria



Julius Adler (1930-)

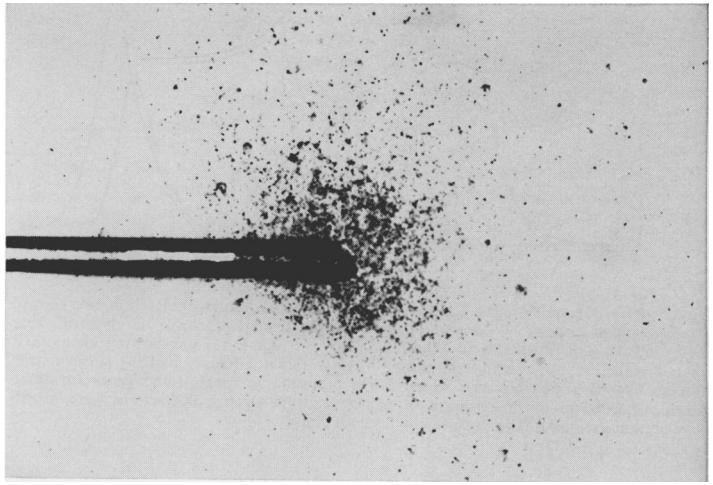
Chemoreceptors in Bacteria

Studies of chemotaxis reveal systems that detect attractants independently of their metabolism.

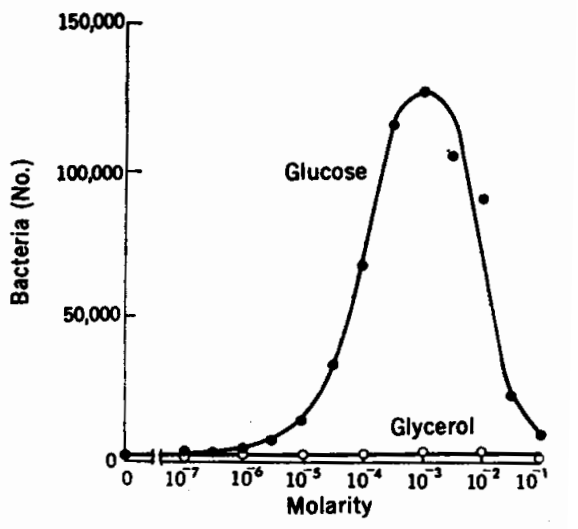
Julius Adler

Summary

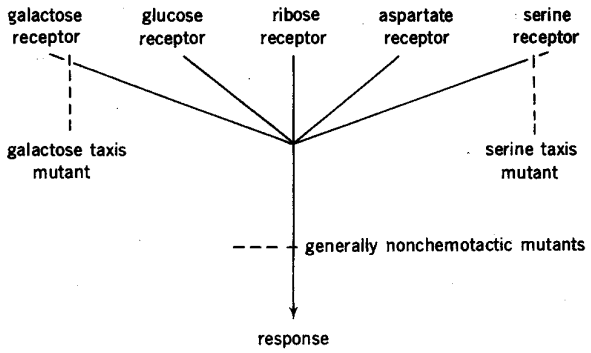
Extensive metabolism of chemicals is neither required, nor sufficient, for attraction of bacteria to the chemicals. Instead, the bacteria detect the attractants themselves. The systems that carry out this detection are called "chemoreceptors." There are mutants that fail to be attracted to one particular chemical or to a group of closely related chemicals but still metabolize these chemicals normally.



E. coli attracted by 2mM Aspartate in capillary
Bacteria enter the capillary during 1h



attraction by Glucose (mM range)



J. Adler, *Science* 166, 1588 (1969).

Chemical guidance of bacteria

Nonchemotactic Mutants of *Escherichia coli*

JOHN B. ARMSTRONG, JULIUS ADLER, AND MARGARET M. DAHL

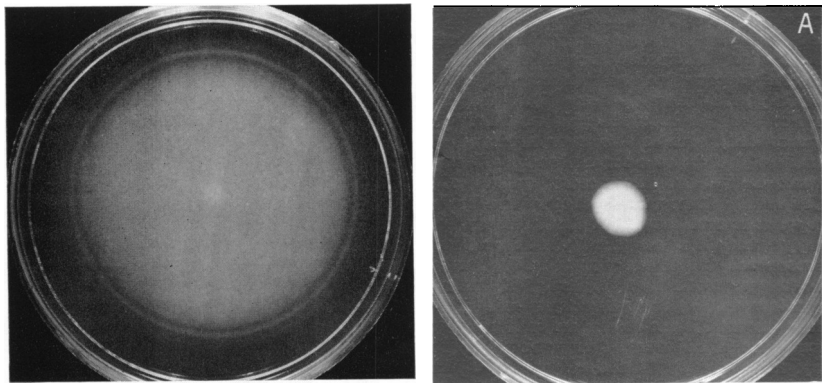
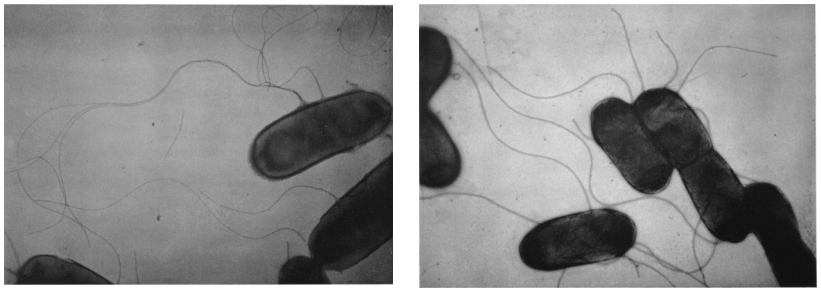


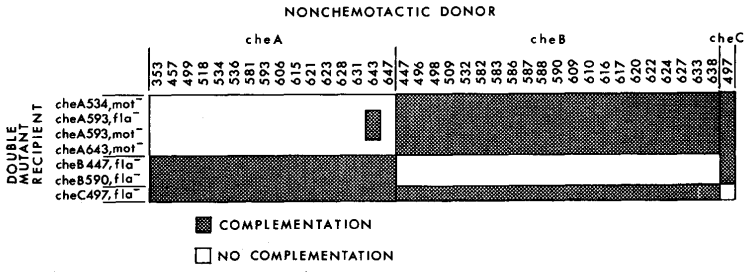
FIG. 1. *Swarming of the parental strain AW330 on a semisolid tryptone plate. A loopful of an overnight tryptone broth culture was spotted on the center of the plate, which was then incubated for 16 hr at 35 C in a water-saturated incubator. If the agar concentration is*

Nonchemotactic mutant *M353*



M353 mutant is flagellated

Armstrong JB, Adler J,Dahl MM *J. Bacteriol*. 93:390– 98 (1967)



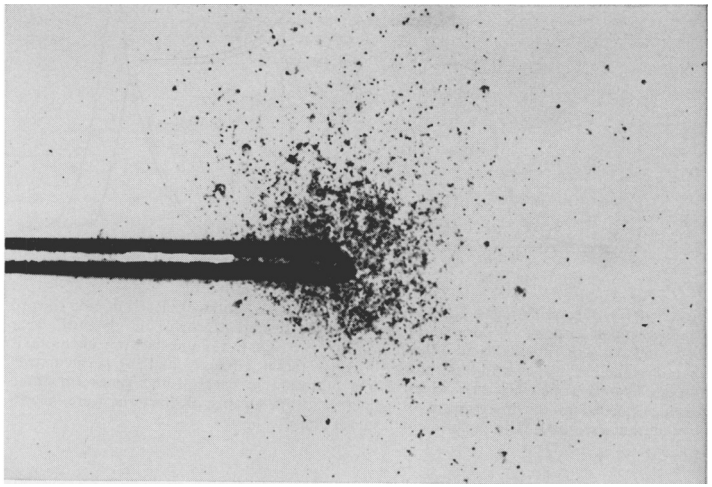
Three categories of Non-chemotactic mutants:

- non flagellated: *fla* - (structure of flagella)
- paralyzed: *mot* - (functioning of flagella)

- normal and motile: *che* -

Armstrong JB, Adler J. *Genetics* 61:61–66 (1969)

Chemical guidance of cell motility



E. coli attracted by 2mM Aspartate in capillary
Bacteria enter the capillary during 1h

Key features of chemotaxis:

- Specificity
- Cell surface sensing (receptors)
- Sensitivity to ratio (gradient)
  but *not difference* in
  concentration of attractant

- **How can cells respond to a chemoattractant gradient?**

Problems: Bacteria can go up an exponential gradient, over 20mm.
For a 2µm cell to detect such a gradient, they would need to detect 0.0001% difference on both ends

Sensitivity to stochastic fluctuations: estimate of 60 molecules of attractant at 1µM on a sampling volume of $1\mu\mathrm{m} \times 1\mu\mathrm{m} \times 0.1\mu\mathrm{m}$ .The standard deviation is $\sqrt{60}$.Yet the response is very accurate and fast (few ms)…

R. Macnab. D.E. Koshland. *PNAS*. 69:2509-2512 (1972)

Two general classes of Mechanisms

- Spatial mechanism: comparison of chemoattractant concentration along cell length

- Temporal mechanism: comparison of chemoattractant at different positions and memory.

Temporal gradient sensing

Daniel Koshland (1920-2007)

The Gradient-Sensing Mechanism in Bacterial Chemotaxis

(temporal gradient apparatus/stopped-flow/*S. typhimurium*/motility tracks/memory)

ROBERT M. MACNAB AND D. E. KOSHLAND, JR.

Department of Biochemistry, University of California, Berkeley, Calif. 94720

- Mechanism: translation through space and temporal gradient detection

- **No gradient:** Cells have similar dynamics at different concentrations of serine

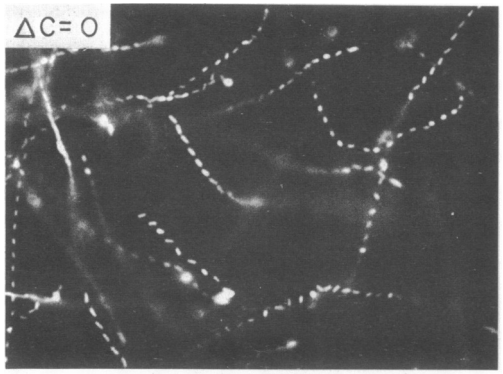
Cells experience a sudden exposure to a new concentration of chemoattractant:

- **Positive Gradient:** 0 to 0.76mM of serine in 200ms
  Cells adopted longer runs
  Their dynamics relaxed to the initial dynamics after 5 min.

- **Negative gradient:** from 1 to 0.24mM. Cells reduced their runs and the dynamics relaxed to the initial one after 12s

Salmonella typhimurium

Temporal projections of bacteria position to see the tracks



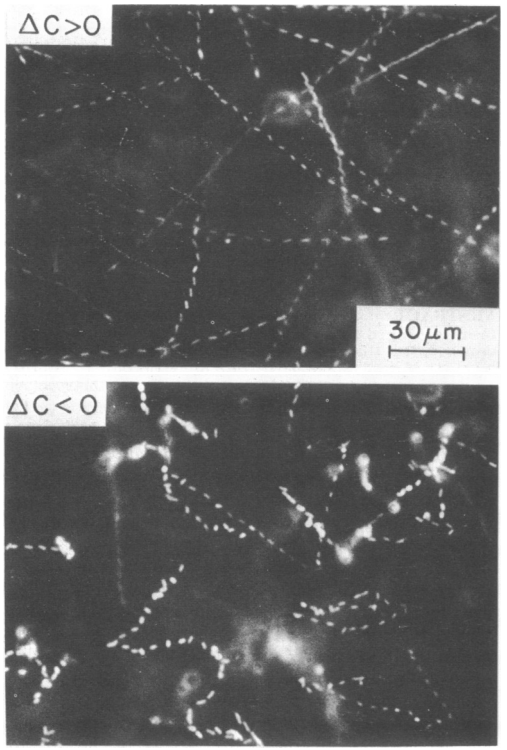


TABLE 1. *Motility of S. typhimurium at constant attractant levels*

Serine concentration (mM)	Velocity of bacteria in sample (μm sec⁻¹)	Overall average velocity (μm sec⁻¹)
0	27.4 ± 4.7	
	29.9 ± 6.0	28.8
	29.0 ± 4.6	
0.01	27.6 ± 4.7	
	28.7 ± 3.5	27.2
	25.2 ± 6.0	
1.0	30.2 ± 4.0	
	29.0 ± 2.9	28.8
	27.1 ± 6.0	

R. Macnab. D.E. Koshland. *PNAS*. 69:2509-2512 (1972)

Chemical guidance of bacteria

Howard Berg (Harvard Univ.)

How to Track Bacteria*

HOWARD C. BERG

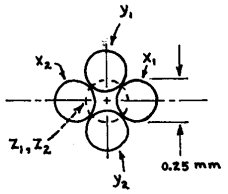
Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80302

(Received 25 January 1971)

A microscope is described which automatically remains focused on individual motile bacteria. The container in which the bacteria are suspended is moved in such a way that the position of a given organism remains fixed; x, y, and z drive signals provide a measure of its displacement relative to the suspension medium. Records are shown of the motion of *Escherischia coli*.

Bacteria swim about 20-50 body length per second so get out of focus within a fraction of a second. Tracking system to within 1 µm

The scene through the binocular is extraordinary. The bacterium being tracked seems to be stuck to the center of the field, turning this way and that trying to free itself, while the other bacteria drift in and out of focus, then to and fro, in apparent synchrony.



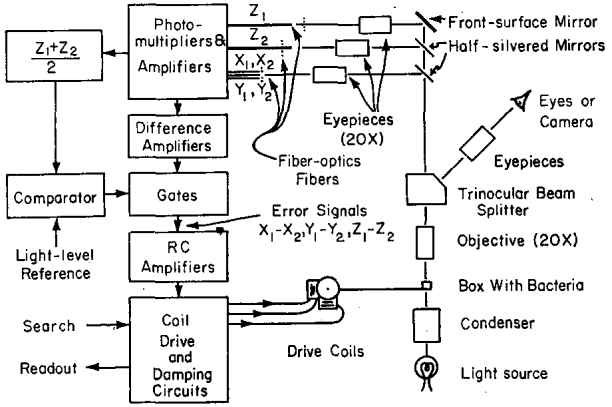


FIG. 1. Schematic diagram of the tracking microscope.

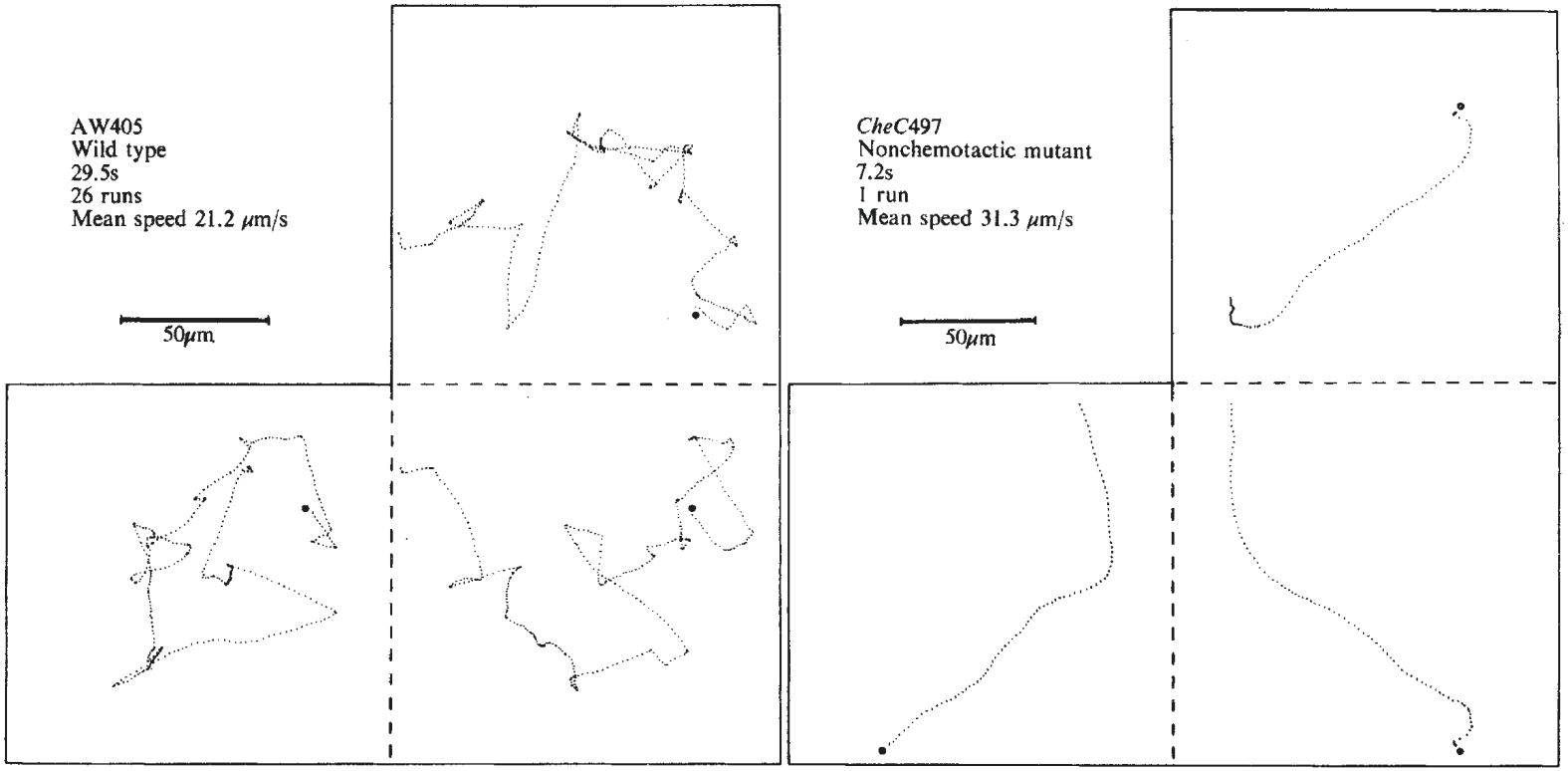
HC. Berg. *Review of Scientific Instruments* 42, 868 (1971)

Random walk of bacteria

Chemotaxis in *Escherichia coli* analysed by Three-dimensional Tracking

HOWARD C. BERG & DOUGLAS A. BROWN

Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80302



Howard Berg and Douglas Brown. 1972, *Nature* 239, 500-504

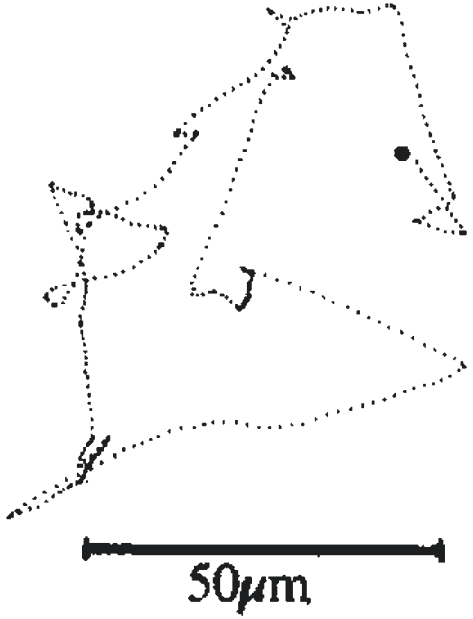
Random walk of bacteria

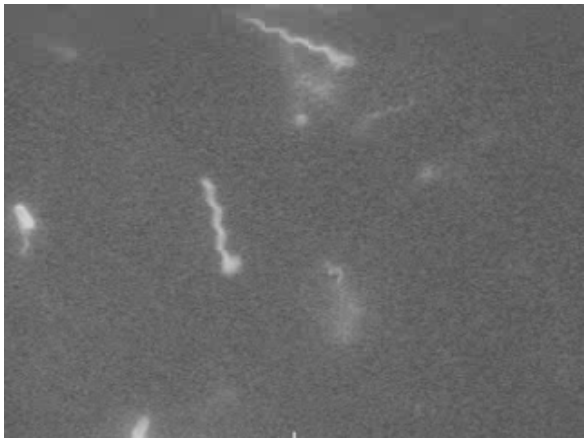
Without chemoattractant

- **Cells follow a succession of runs and « tumble »**

Runs are at average velocity 14µm/s and the mean run length is about 1s. During tumbles, cells are immobilized. Tumbles length is about 0.1s

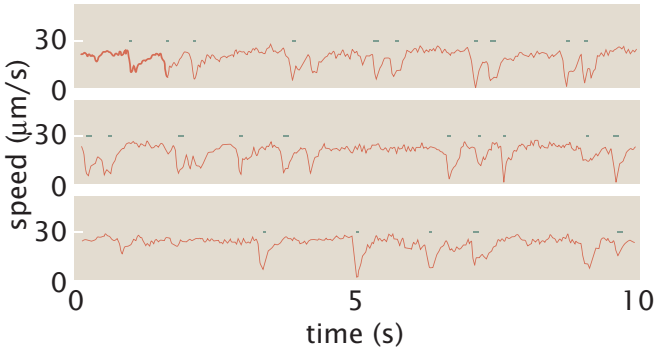
As cells resume movement, they adopt a new, nearly random trajectory (62±26°)

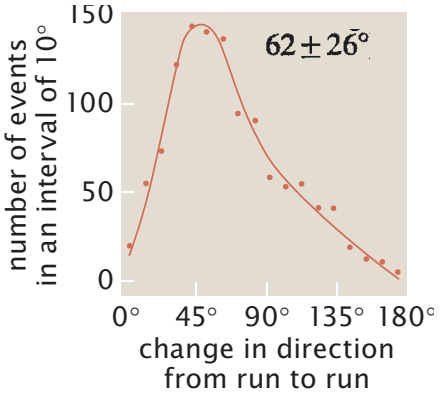




- **Runs and tumbles occur at random (Poisson statistics)**

For a given organism in a given environment, the probability per unit of time to stop a run or a tumble is a constant





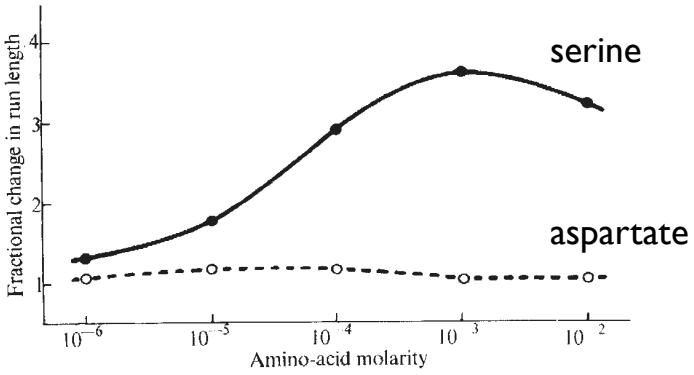
Howard Berg and Douglas Brown. *Nature* 239, 500-504 (1972)

Random walk of bacteria

With *uniform* chemoattractant

In presence of *eg.* serine, the distribution of runs is exponential, runs occur still at random **But runs are significantly longer and tumbles are suppressed.**

In other words, with high chemoattractant, cells tend to continue in the same direction and reduce the frequency of tumbling



Howard Berg and Douglas Brown. *Nature* 239, 500-504 (1972)

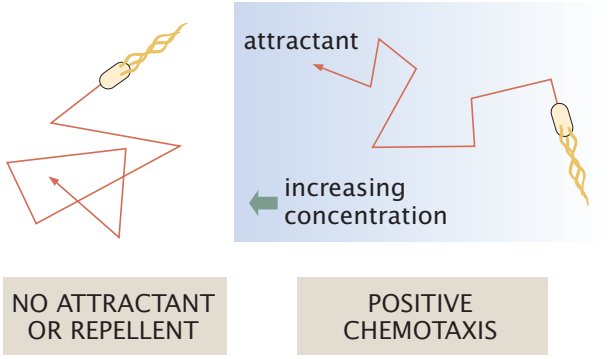
Biased random walk of bacteria

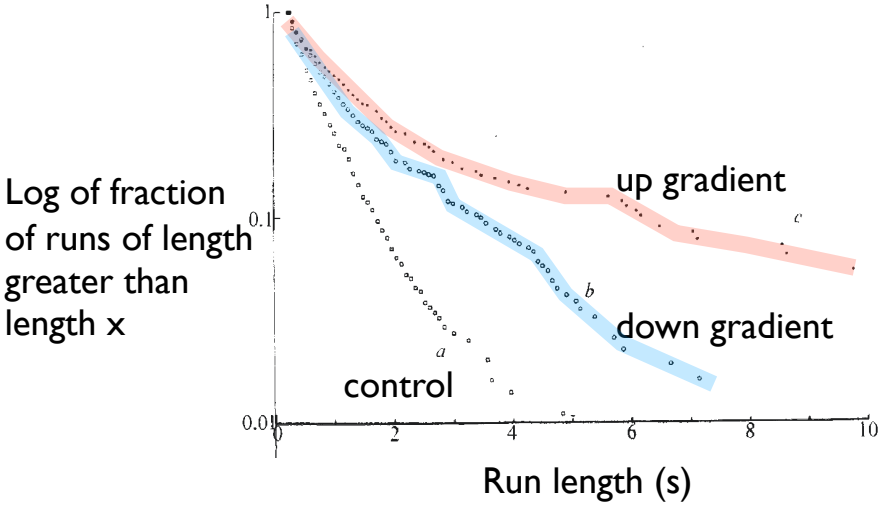
Gradient of chemoattractant

- **Runs up and down the gradient are very different:**
  — up the gradient: runs are longer than is expected from the concentration dependence of the runs (ie. tumbles are postponed)
  — down the gradient runs occur at probability expected for solution of same isotropic concentration

Attractant	Serine	Serine
Net displacement of runs	Up	Down
Mean concentration (μM)	10.0 ± 2.8	9.2 ± 2.6
Mean run length (s)	2.19 ± 3.43	1.40 ± 1.88
Mean run length expected from the control run length (Table 2) and the concentration dependence (Fig. 5) (s)	1.48	1.45

- **The chemical signal is sensed and acted on only when the cell swims up the gradient**
- **Moving up the gradient reduces the tumbling frequency**
- **Cells spend more time going up the gradient than down, so they go up the gradient**

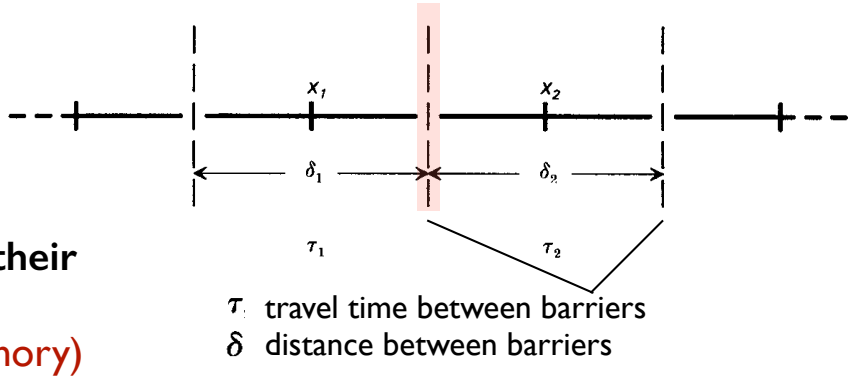




Howard Berg and Douglas Brown. *Nature* 239, 500-504 (1972)

How can Chemotaxis work?

1D theoretical model of stochastic motion:
Semipermeable barriers that reflect ½ of particles (eg. bacteria), and let ½ pass through



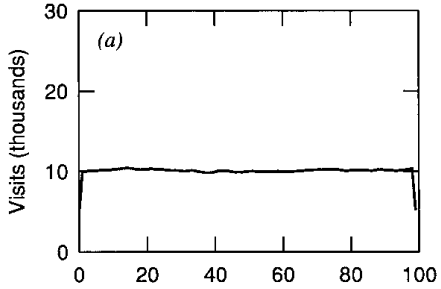
This models the idea that particles change randomly their trajectory every time τ and distance δ
Their motion is defined locally in space and time (no memory)

Flux: $J = -(1/4)[C(x_2)(\delta_2/\tau_2) - C(x_1)(\delta_1/\tau_1)]$. δ/τ: velocity

- **Case 0**: δ and τ are constant. Fick's law $J = -D(\partial C/\partial x)$, with diffusion coefficient $D = \delta^2/4\tau$
  At equilibrium C is uniform

Let us consider cases where D **changes in space (** δ and/or τ vary in space**):**

- **Case 1**: velocity δ/τ is constant, but δ and τ vary in space
  we still have $J = -D(\partial C/\partial x)$, $D = \delta^2(x)/4\tau(x)$ *is* not constant in space



Monte Carlo simulations

Thus, whatever the distribution of barriers, **provided that velocity is constant** the distribution of particles at equilibrium will always be uniform

If bacteria have a uniform velocity, changing in space the probability of changing direction (tumbling) will not lead to spatial accumulation of cells. So if an attractant were to simply change the tumbling frequency there would be no chemotaxis.

Schnitzer M, Block S, Berg HC, Purcell E. *Symp. Soc. Gen. Microbiol.* 46:15–34 (1990)

How can Chemotaxis work?

Let us consider cases where D **changes in space:**

- **Case 2**: distance δ is constant $J = -D(\partial C/\partial x) - C(\partial D/\partial x) = -\partial(DC)/\partial x,$ $D = \delta^2/4\tau(x)$

  At equilibrium DC is uniform, and C is inversely proportional to D
  Therefore, particles accumulate where their velocity is lowest

- **Case 3**: time τ is constant $J = -D(\partial C/\partial x) - C(\partial D/\partial x)/2.$ $D = \delta^2(x)/4\mathrm{t}$

  At equilibrium $D^{1/2}C$ is uniform, and C is inversely proportional to $D^{1/2}$
  Therefore, particles accumulate where their velocity is lowest

- **Case 4:** all parameters vary in space $J = -(\delta/4)[v(\partial C/\partial x) + C(\partial v/\partial x)],$ (δ/τ)

  At equilibrium, the density of particles is still inversely proportional to velocity

When speed is not constant, cells accumulate in regions of low speed
When speed is constant, cells remain uniformly distributed whatever the frequency of tumbling as a function of stimulus (klinotaxis).

Schnitzer M, Block S, Berg HC, Purcell E. *Symp. Soc. Gen. Microbiol.* 46:15–34 (1990)

Chemotaxis at Low Reynolds number

- A bacterium needs to explore different chemical environment to find the most nutrient rich one (10^9 glucose molecules are needed for 1 cell cycle in 1h)
- **What is the best strategy to read and explore the environment?**

- Stirring liquid versus Diffusion?
  — At low Reynolds number, stirring is very slow/inefficient compared to diffusion
  Cells cannot shake off the liquid around

The transport in and out of molecules to a bacterium is largely governed locally by diffusion.

Diffusion: $\tau_D = (l^2 / D) \approx 1\ \text{ms}\ (D = 10^3 \mu\text{m}^2/\text{s})$

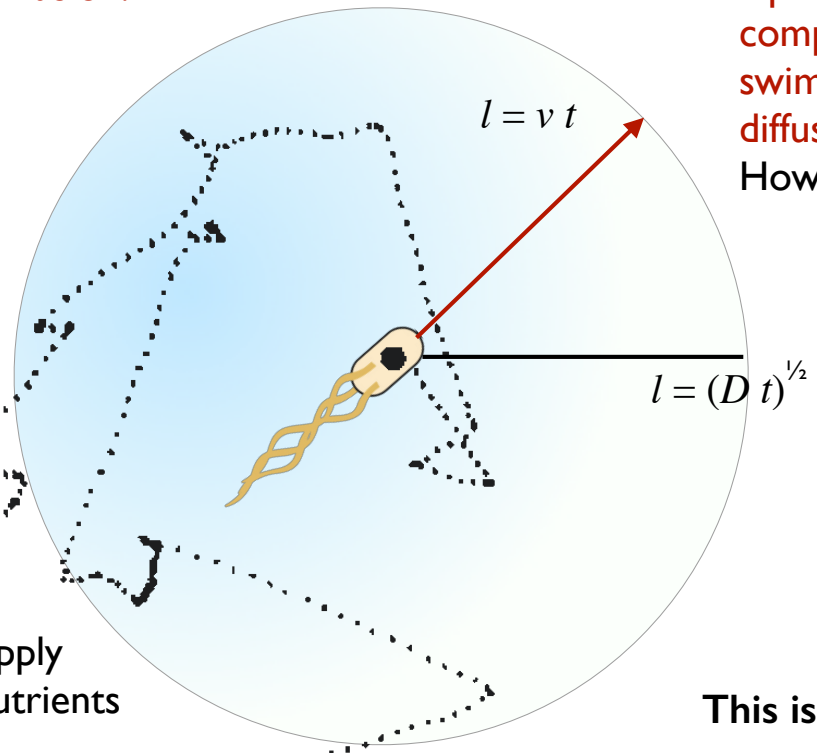
Swim: $\tau_S = l / v \approx 100\ \text{ms}$

$\tau_D << \tau_S$

Peclet number: $P_e = \frac{\tau_D}{\tau_S} = l.v\ /D$

$P_e \approx 10^{-2}$

>>Swimming does not increase food supply
Strategy: wait for diffusion to bring nutrients



To find a new environment with a potentially different chemical composition, *E. coli* needs to swim far enough to outrun diffusion within time *t*.
How far is this?

$v\ t > (D\ t)^{1/2}$

$t > D\ /v^2$

$t > 1\ \text{s}$ For $30\mu\text{m/s}$ $D = 10^3 \mu\text{m}^2/\text{s})$

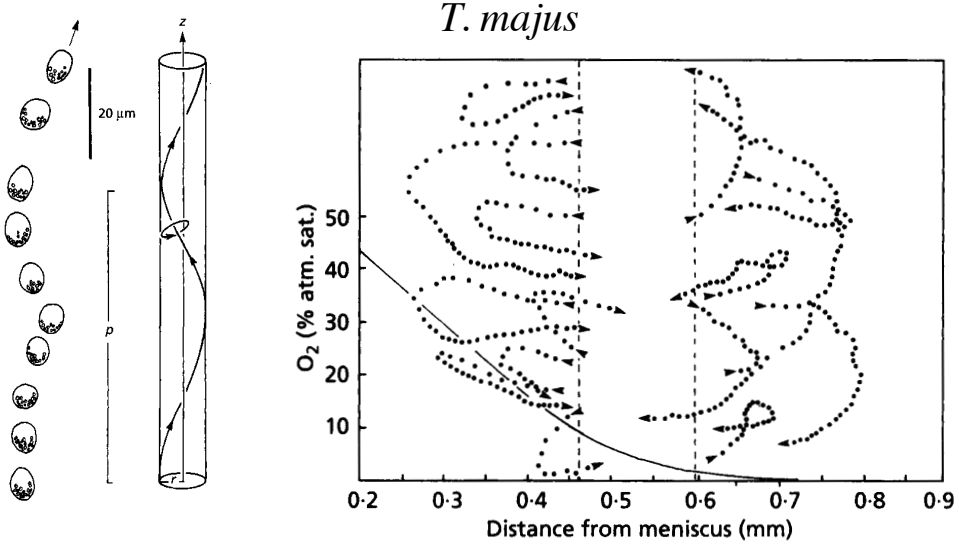
Therefore $l > 30\mu\text{m}$

This is the typical length of a run

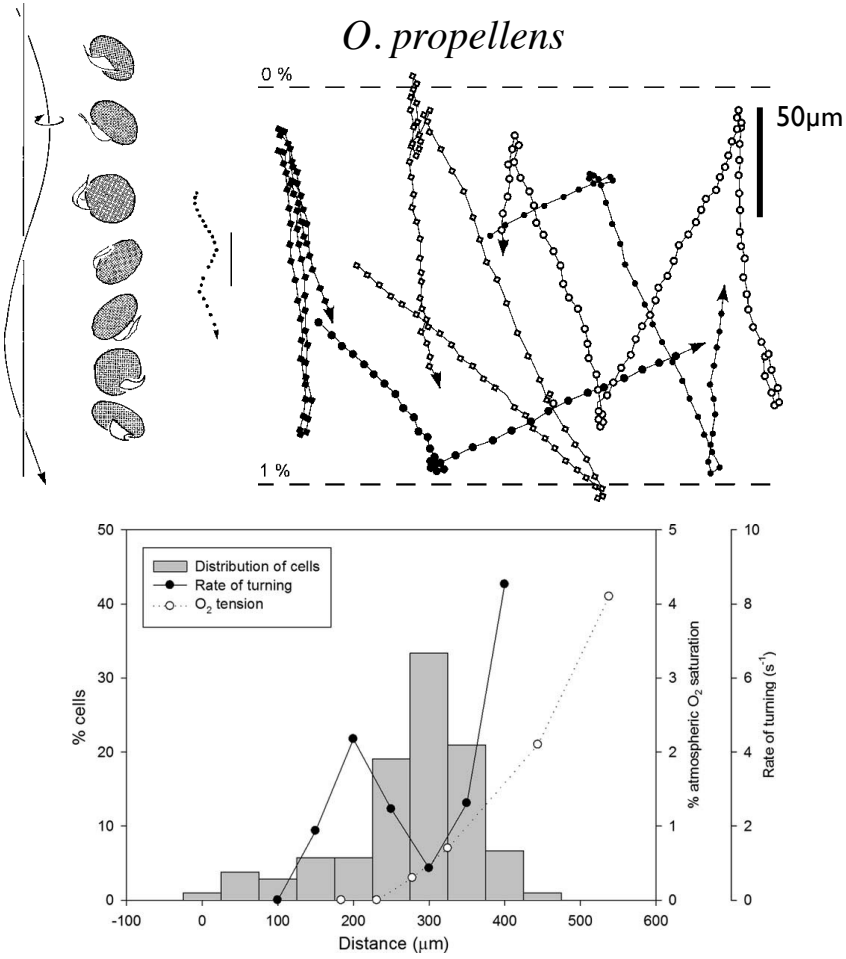
E. Purcell. *American Journal of Physics* 45, 3 (1977)

Chemotaxis at Low Reynolds number

- Oxygen sensing bacteria swim very fast: at speeds ranging from 100-1000µm/s.
- These bacteria concentrates in sediments at a very specific oxygen pressure
- *Thiovulum majus* and *Ovobacter propellens* swim at 600-700µm/s along 1D gradient of O_2.
- They follow180° U-turns when the oxygen concentration falls or rises above a value.



Fenchel, T. *Microbiology* 140, 3109– 3116. (1994)



- At such high speeds, diffusion is almost outcompeted by swimming.

Peclet number: $P_e = \frac{\tau_D}{\tau_S} = l.v\,/D$

length: 4 μm
speed: 700-1000 μm.s^{-1}
D_{O_2} = 2. 10^{-5} μm^2.s^{-1}

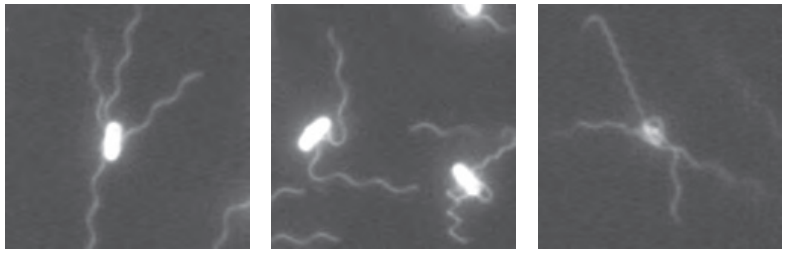
$\rightarrow$ $P_e \approx 1.4 - 2$

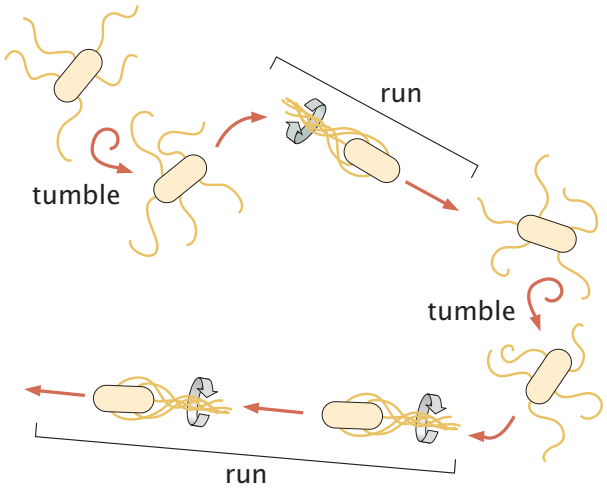
Fenchel T. and Thar R. *FEMS Microbiology Ecology* 48:231–238 (2004)

Chemoreceptor physiology

Tumble frequency

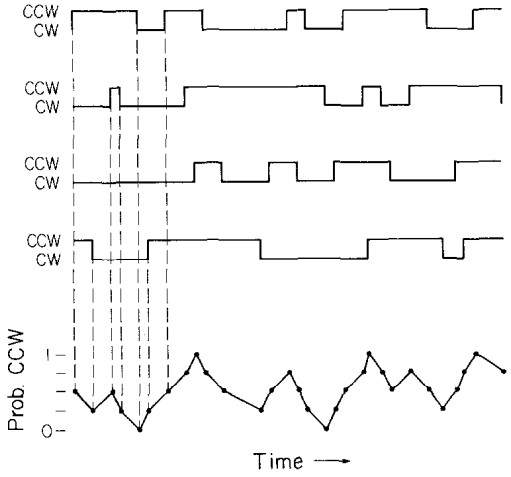
- **Runs and tumbles are caused by a rotational switch of flagella**
- Runs: counterclockwise (CCW) rotation leads to filament bundling
- Tumbles: clockwise (CW) rotation causes dissociation of filaments and cessation of cell movement



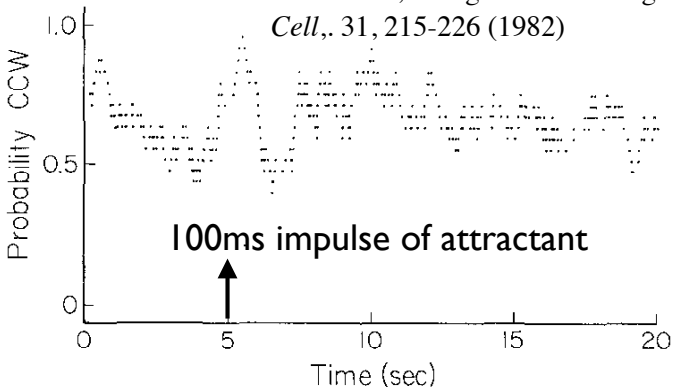


- The swim of an *E. Coli* can be characterized by the tumbling frequency.
- The presence of a chemoattractant reduces the tumbling frequency
- This can be measured by the **rotational bias of the motor** based on single cell recordings

Probability is average of individual tracks where +1 for CCW and 0 for CW rotation



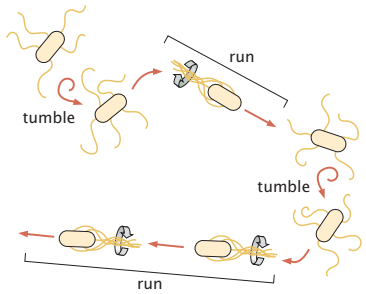
SM. Block, J. Segall and H. Berg. *Cell*,. 31, 215-226 (1982)



Average of 25 recordings in single cell

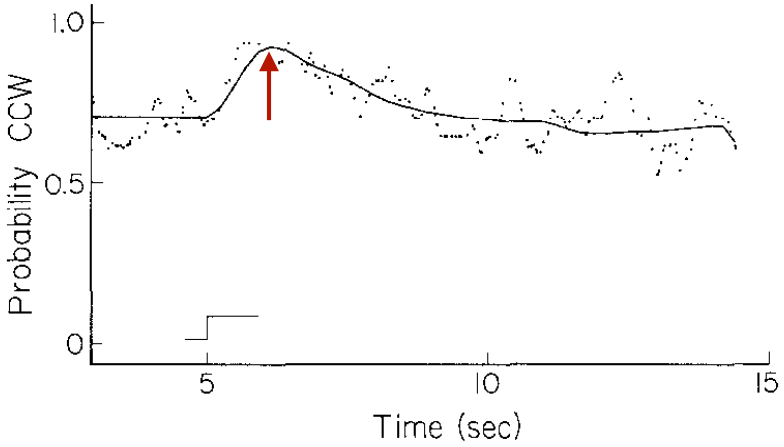
Chemoreceptor physiology

Regulation of tumbling frequency

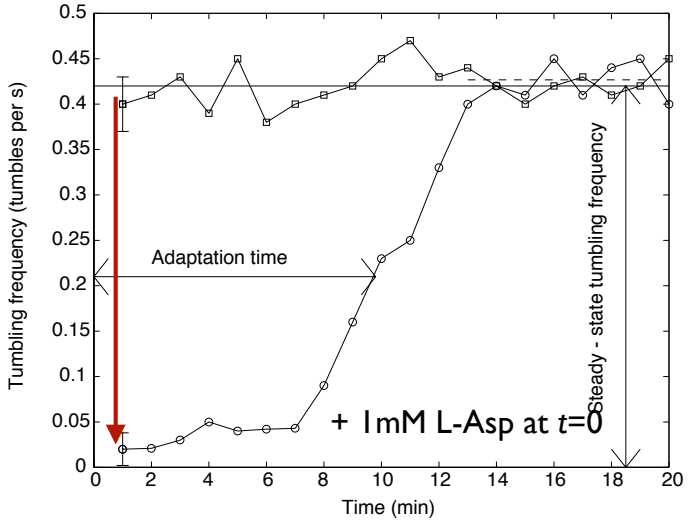


- **Chemoattractant inhibits intrinsic frequency rotational switch of motor**

A small step of chemoattractant increases CCW rotational bias, and reduces tumbling frequency



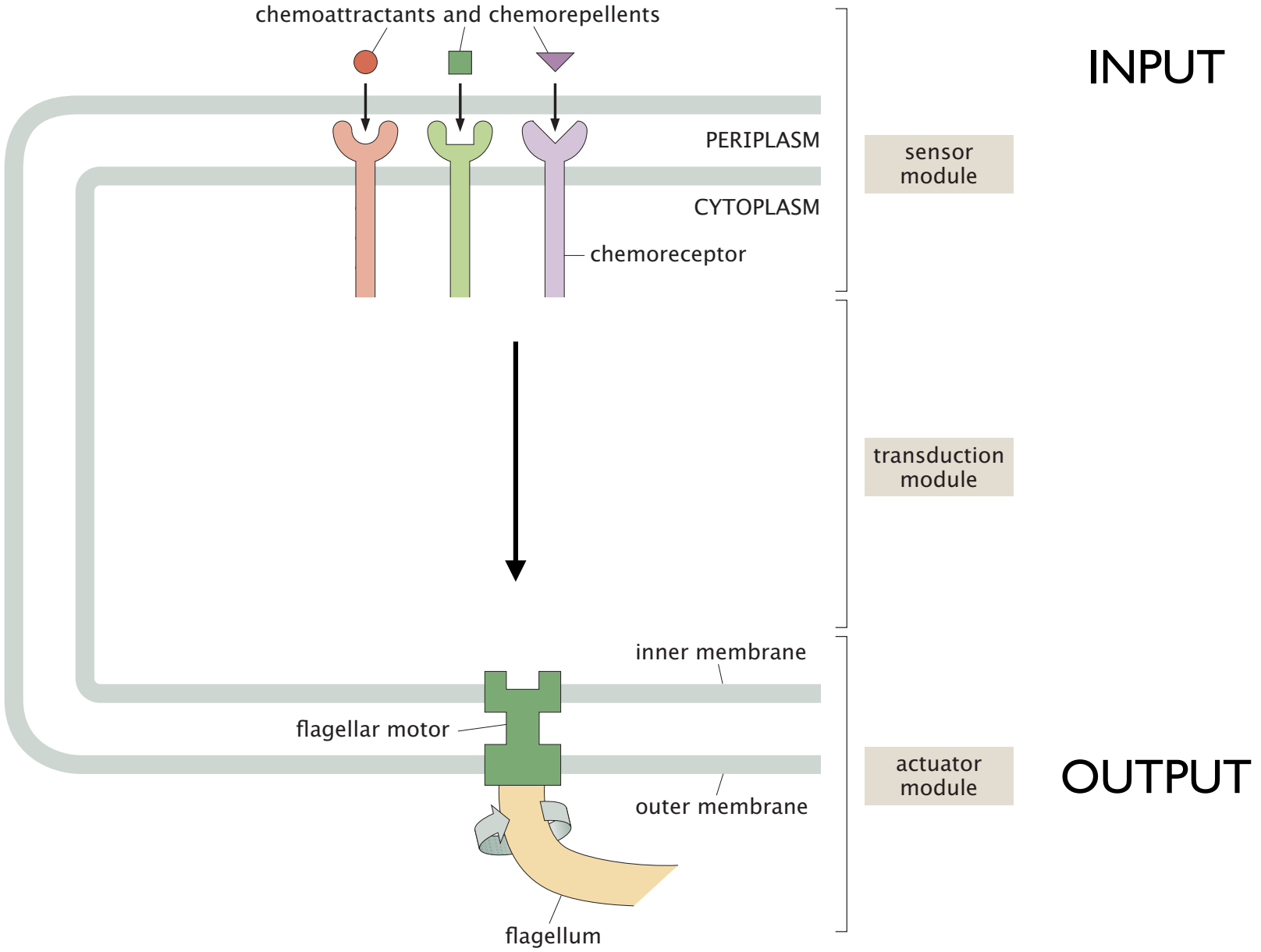
SM. Block, J. Segall and H. Berg.
Cell,. 31, 215-226 (1982)



unstimulated cell

Alon, U, Surette, MG, Barkai, N, &
Leibler, S *Nature* 397, 168. (1999)

Molecular circuit driving chemotaxis



R. Phillips, The Molecular Switch: signaling and allostery. *Princeton Univ. Press*. 2020

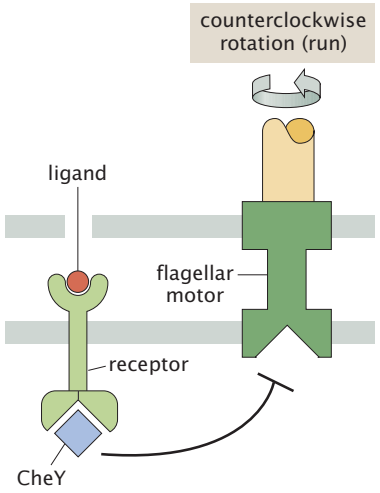
Chemoreceptor physiology

Regulation of tumbling frequency

- **This requires the « messenger» CheY**

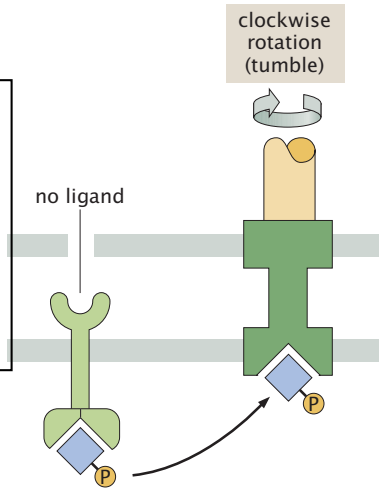
— ligand binding: CheY is unphosphorylated and does not signal: Receptor is OFF, rotation is CCW
— no ligand: CheY is phosphorylated, signals: Receptor is ON, rotation is CW
CheY-P binds the motor and reverses rotation

INPUT	Ligand: bound Receptor OFF
OUTPUT	Motor CCW Run



Ligand: no
Receptor ON

Motor CW
Tumble



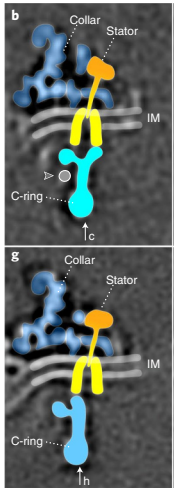
R. Phillips, J. Kondev, J. Thériot and H. Garcia. *Physical Biology of the Cell*. *Garland* 2012

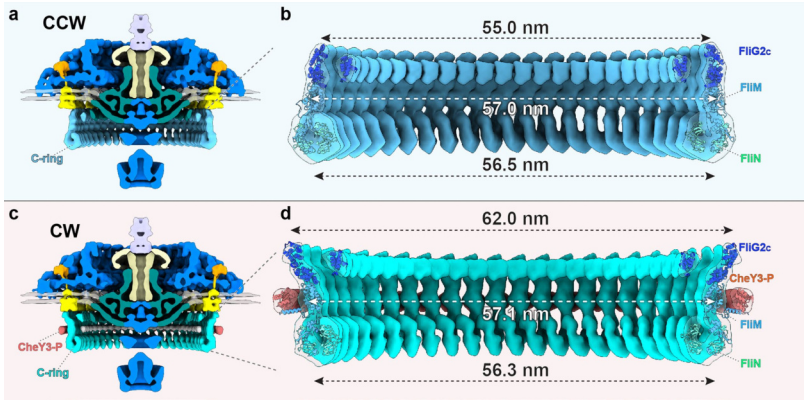
Mechanism of motor rotational switch

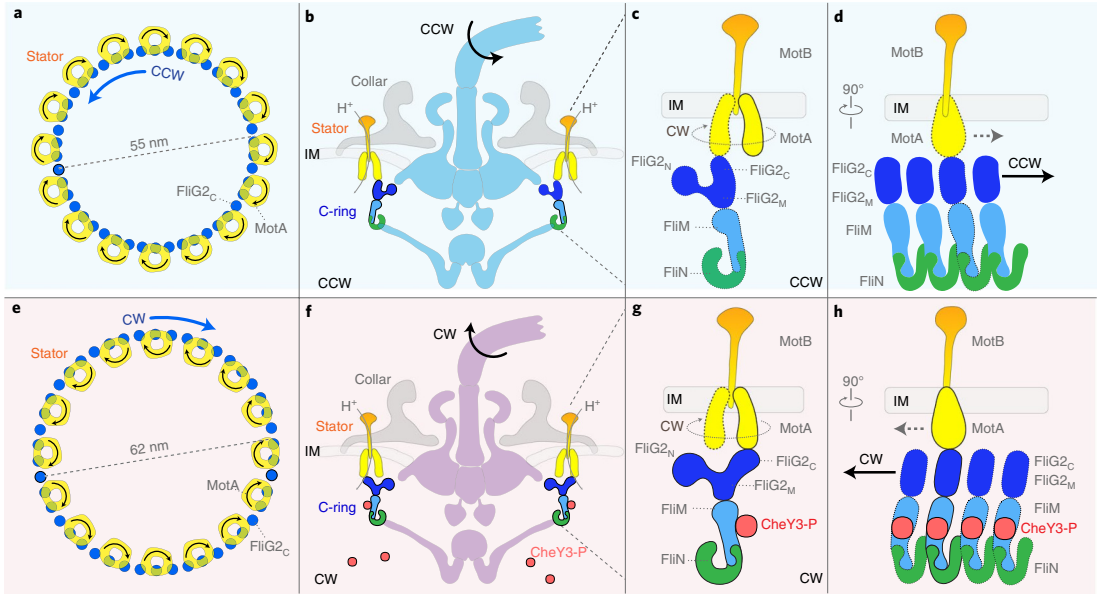
Regulation of tumbling frequency

CheY-P has high affinity for FliM subunit of rotor

Conformational change in FliG2 induced by CheY-P binding to FliM changes the interaction between C-Ring and MotQA stator units

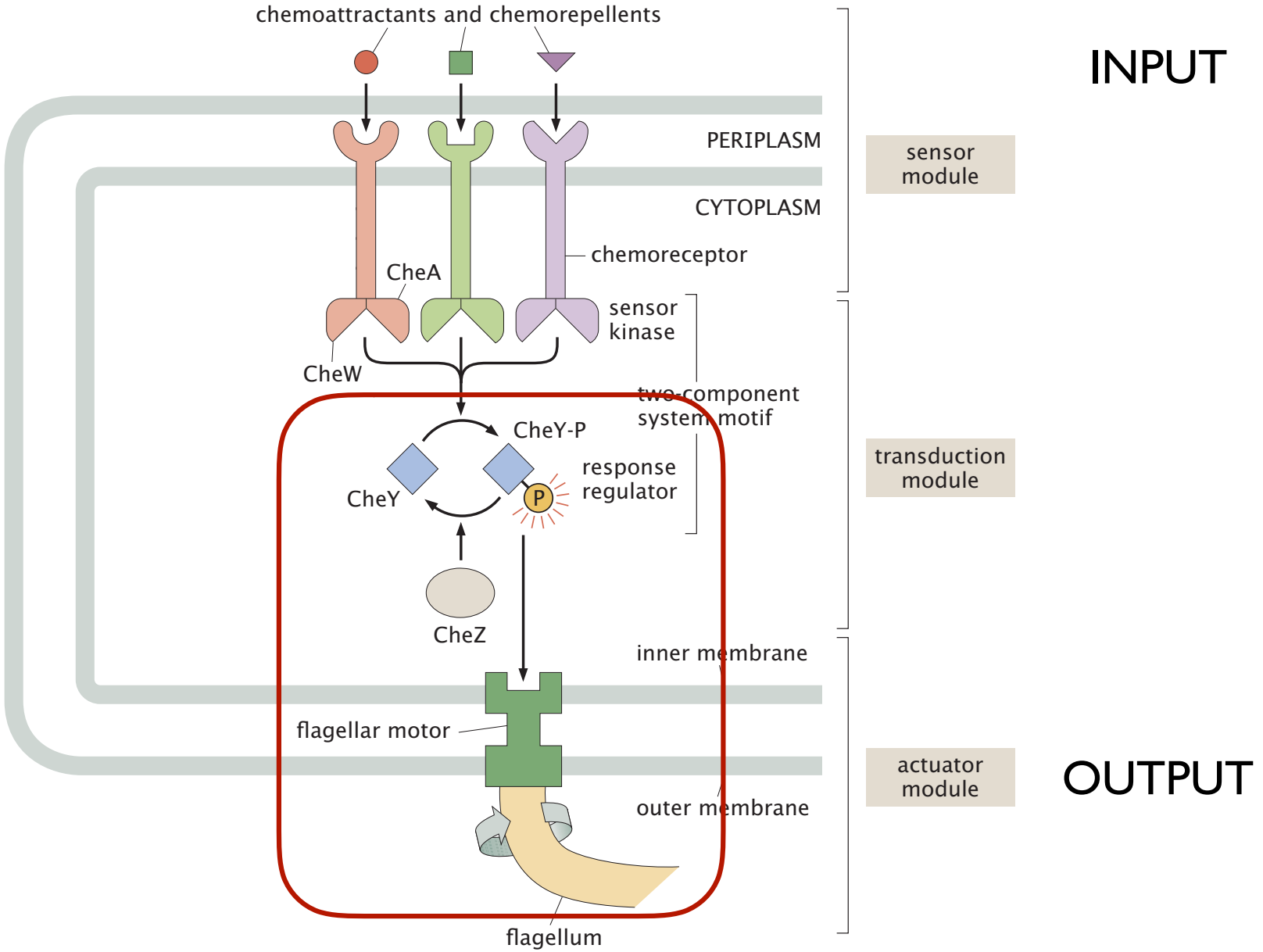






Y. Chang et al and J. Liu. *Nature Structural & Molecular Biology*. 27:1041–1047 (2020)

Molecular circuit driving chemotaxis

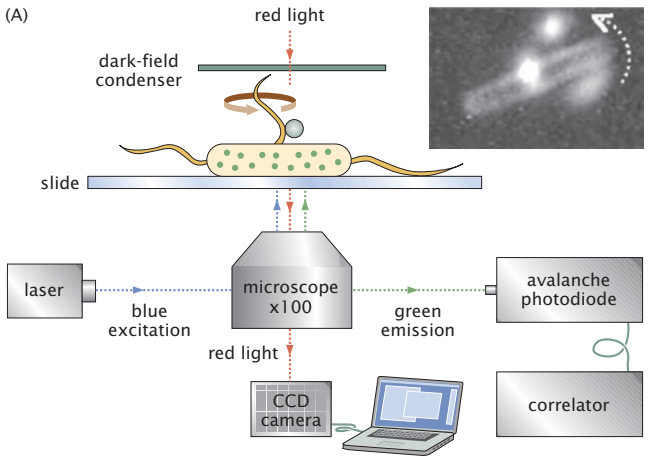


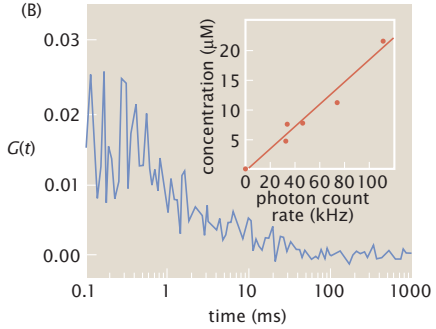
R. Phillips, The Molecular Switch: signaling and allostery. *Princeton Univ. Press*. 2020

Motor ultrasensitivity

- **Correlation between Motor rotational bias and CheY-P concentration**

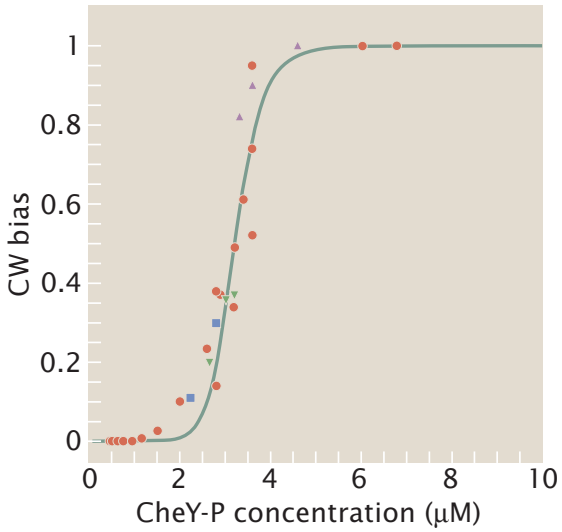
FCS: CheY-GFP concentration
Rotation was monitored with latex beads at tip of flagella

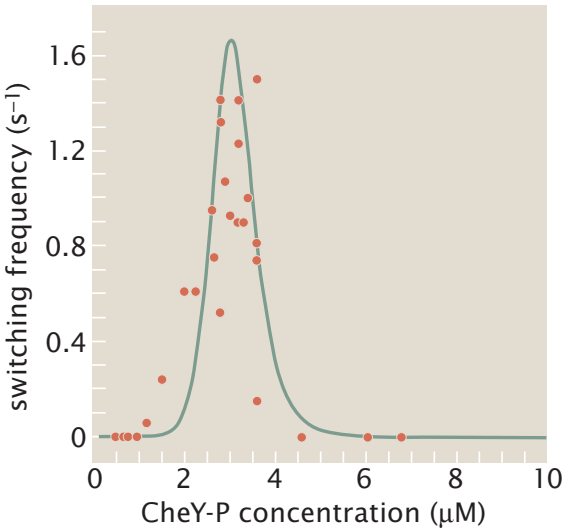




- **Ultrasensitive response of motor to CheY-P:**

Steep Input-Output relation
Hill function: coefficient n≈10
Kd ~ 3μM





P. Cluzel, M. Surette, and S. Leibler, *Science*, 287:1652, (2000)
R. Phillips, J. Kondev, J. Thériot and H. Garcia. *Physical Biology of the Cell*. *Garland* 2012

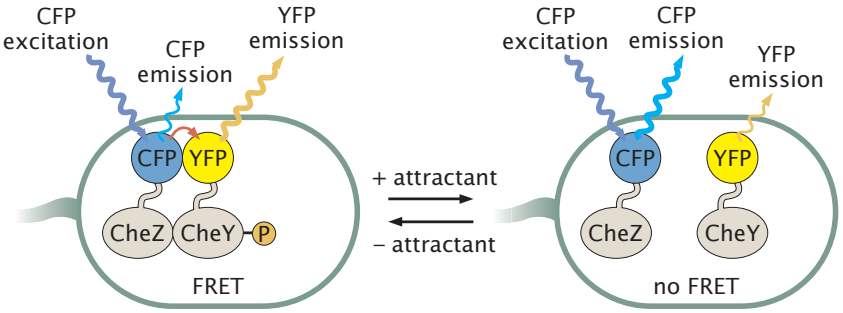
Motor ultrasensitivity

Measuring CheY-P concentration

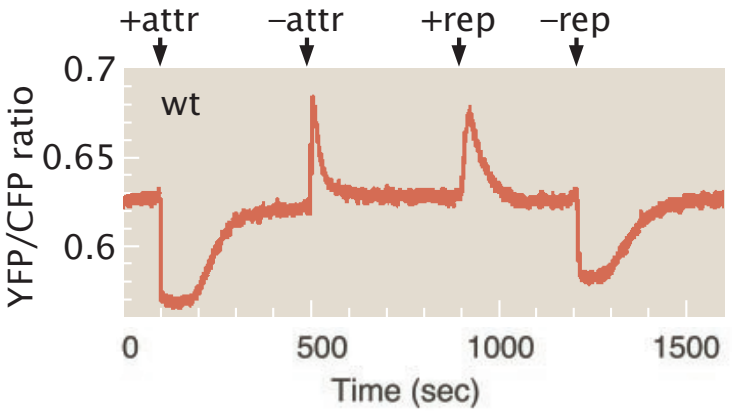
Fluorescence Correlation Spectroscopy to measure CheY-GFP concentration as a proxy for CheY-P

P. Cluzel, M. Surette, and S. Leibler, *Science*, 287:1652, (2000)

- CheZ is a phosphatase that binds to and hydrolyses CheY-P
- At steady state, the rates of phosphorylation and hydrolysis are equal, so the activity of the receptor complex is proportional to the concentration of the CheY-P/CheZ complex



- Receptor binding to chemoattractant represses CheY-P activation so FRET level is reduced within ~1 s
- This is followed by slow (minutes time scale) recovery of FRET, ie. CheY-P levels increase to base line and resets CW bias: adaptation



Sourjik, V. & Berg, H. C. *Proc. Natl Acad. Sci. USA* 99, 123–127 (2002).

Motor ultrasensitivity and Motor adaptation

- **Problem**:

1. The output of the chemotaxis network, the motor, is ultra sensitive: operational value of CheY-P is ~3μM
2. Yet, the concentration of CheY-P is variable between cells.

- **Question**:

How do cells adjust the motor to the variable concentration of CheY such that the chemotactic response is always sensitive?

- **Response**:

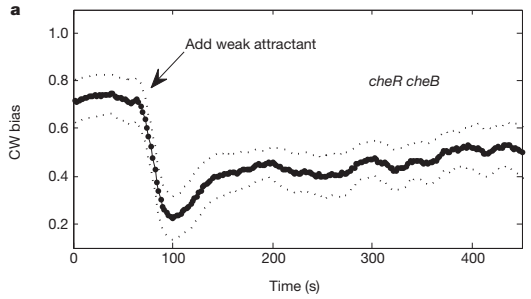
No feedback of motor output on kinase activity.

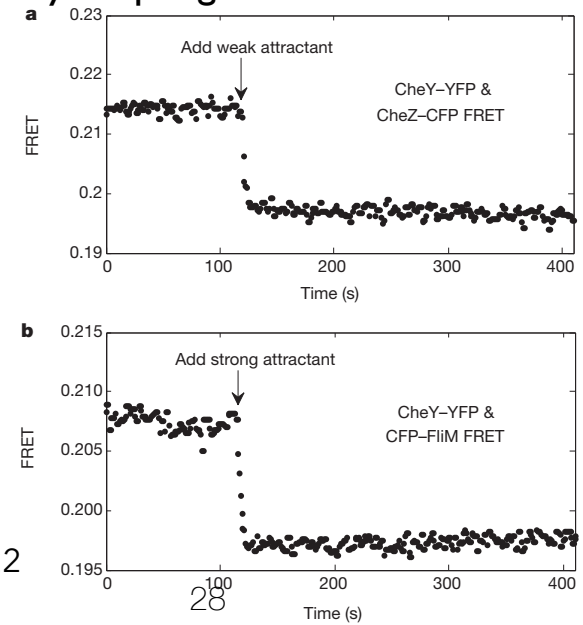
The motor adapts its operating point to the output of chemotactic receptor complex, the kinase activity.

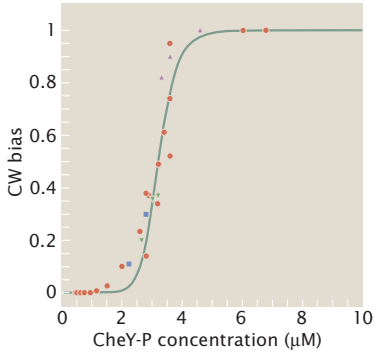
- **Experimental test:**

Use *cheBcheR* mutant cells that are poorly adapting and thus do not maintain a constant steady-state concentration of CheY-P

Stimulated *cheBcheR* cells keep a lower CheY-P concentration







J. Yuan et al and H. Berg. *Nature* 484:233-236 (2012)

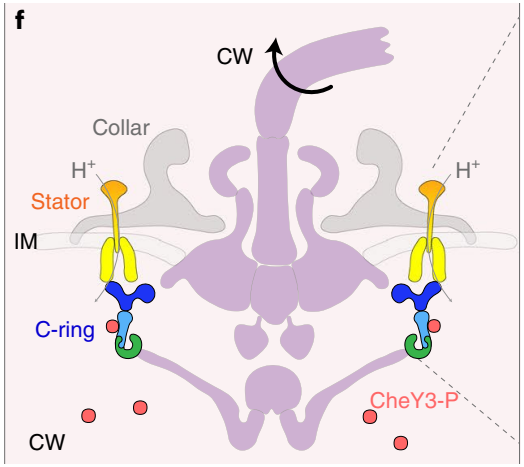
Motor ultrasensitivity and Motor adaptation

- **Question:**

How do cells adjust the motor to the variable concentration of CheY such that the chemotactic response is always sensitive?

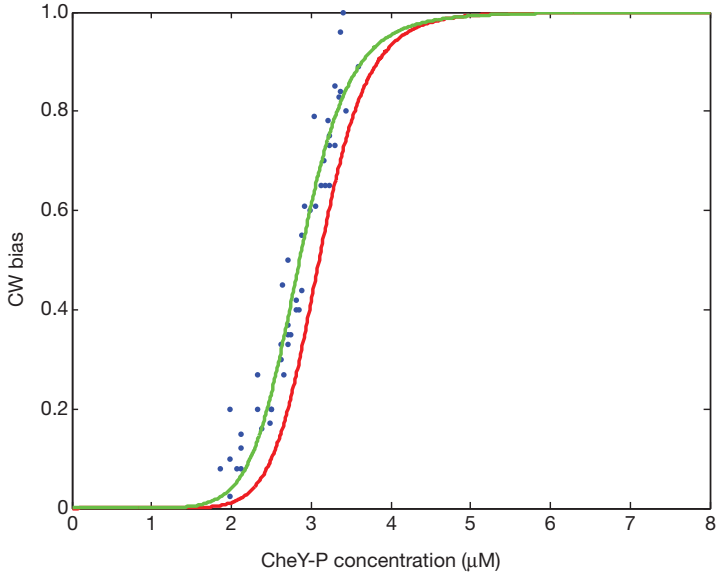
- Response:

No feedback of motor output on kinase activity. Instead, the motor itself adapts to kinase activity by adjusting the number of FliM subunit in the rotor: **A lower concentration of CheY-P causes an increase in FliM units so cells increase the number of CheY-P binding sites, ie. their sensitivity to CheY-P.**

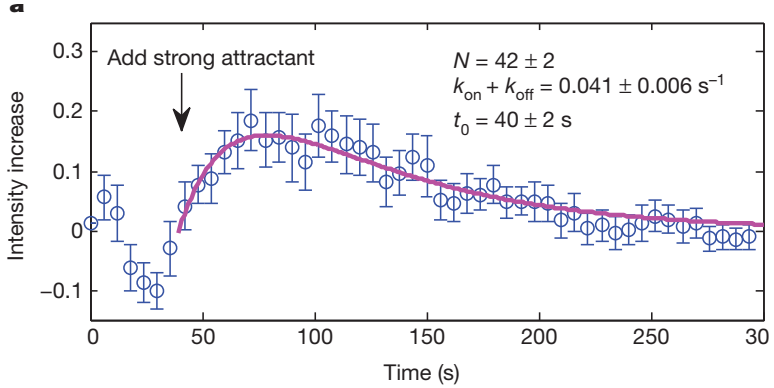


Y. Chang et al and J. Liu. *Nature Structural & Molecular Biology*. 27:1041–1047 (2020)

Comparison of CW bias as a function of CheY-P before stimulation with attractant (red) After cells were stimulated and had partially adapted (green) Cells become more sensitive fo CheY-P (2.7µM instead of 3.1µM). Can be modeled by increase of FliM subunits from 34 to 36.



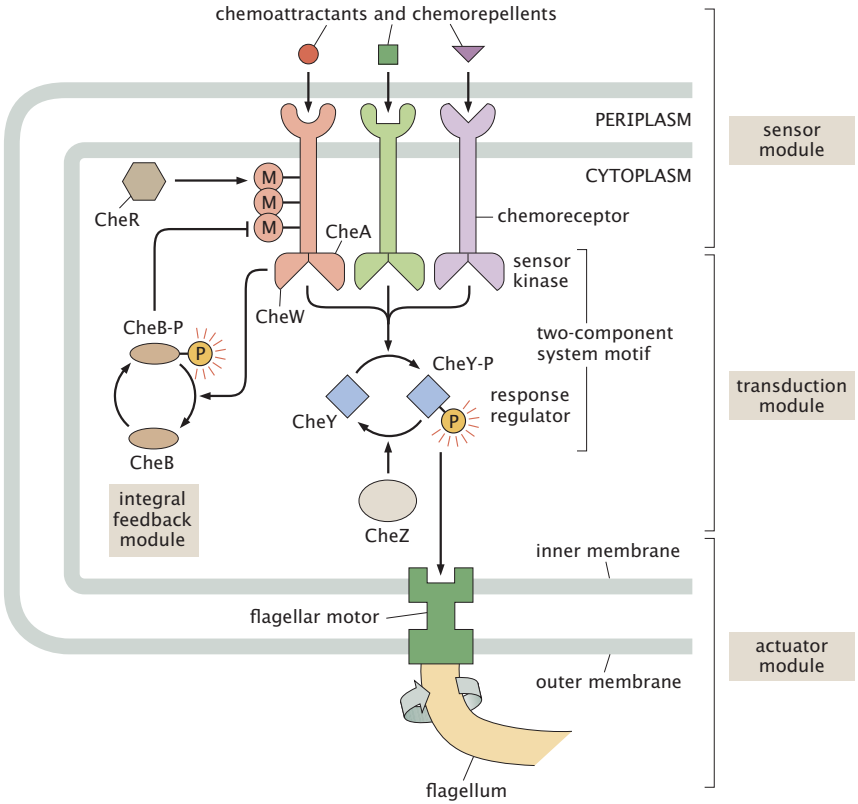
FliM concentration increases in response to attractant



J. Yuan et al and H. Berg. *Nature* 484:233-236 (2012)

Chemoreceptor physiology

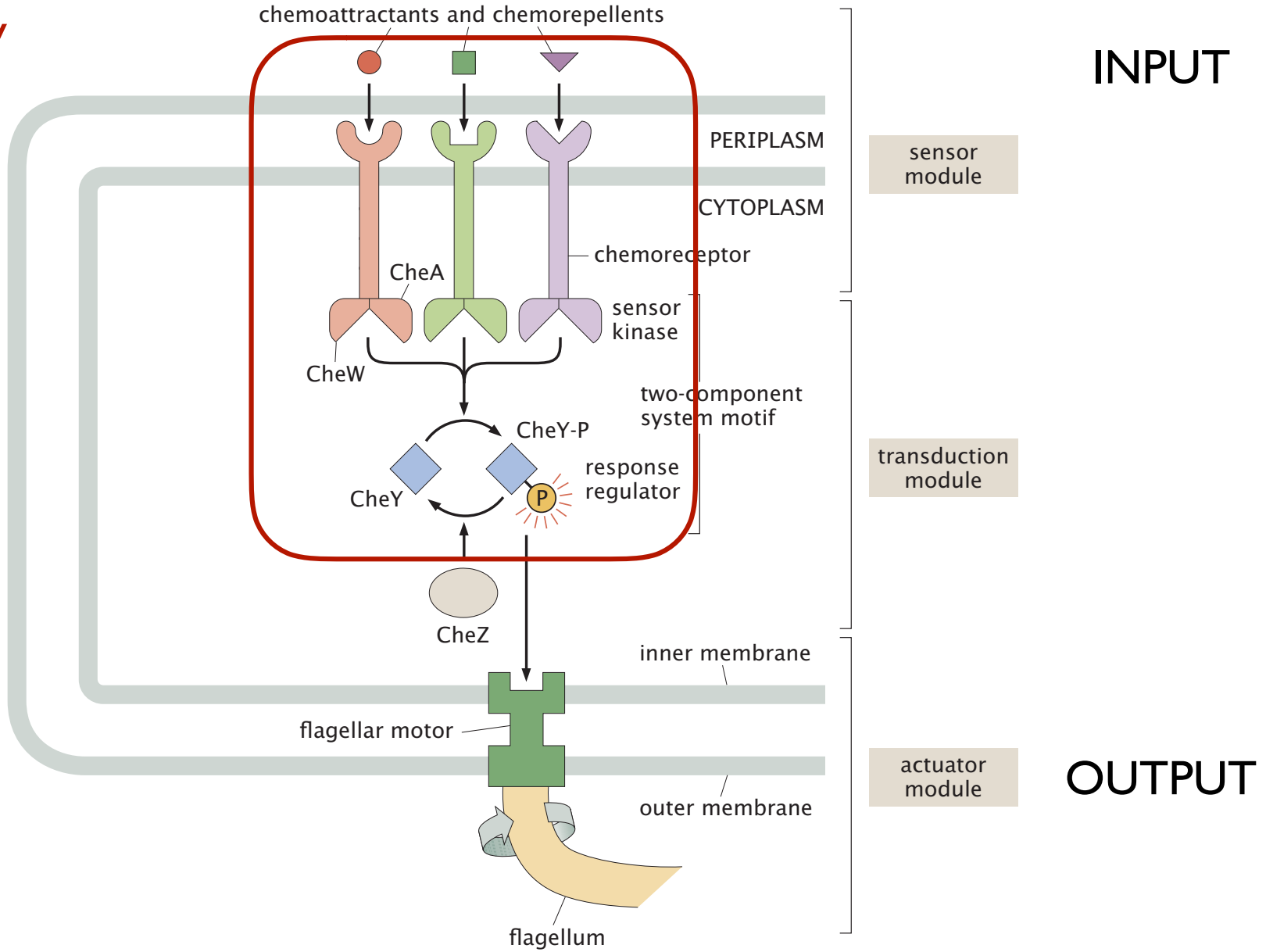
Key properties of chemotactic network



- Sensitivity - Gain : output/input ratio
- Adaptation: reset after input
- High amplitude range

Molecular circuit driving chemotaxis

- Sensitivity

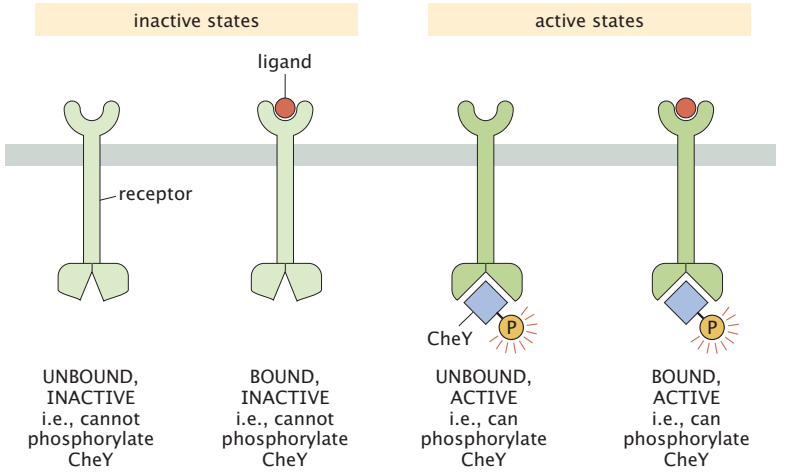


R. Phillips, The Molecular Switch: signaling and allostery. *Princeton Univ. Press*. 2020

Two-state allosteric model of chemotaxis

P_{active} probability that the receptor is ON

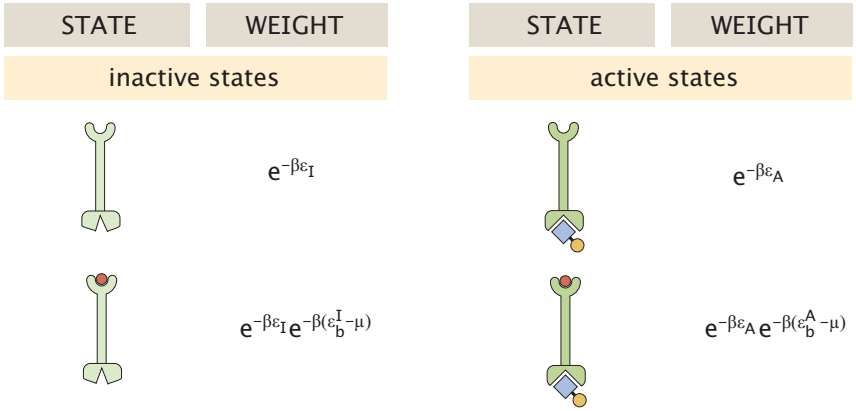
$$p_{active} = \frac{1}{1 + e^{-\beta(\varepsilon_I - \varepsilon_A)} \frac{1 + (c/K_I)}{1 + (c/K_A)}}.$$



$\Delta\varepsilon = \varepsilon_I - \varepsilon_A$: the energy difference between the "inactive" and "active" states of the receptor in the absence of ligand.

K_I, K_A: affinities of the receptor for the ligand in the inactive and active states.

$$K_A = c_0 e^{\beta(\varepsilon_b^A - \varepsilon_{sol})} \qquad K_I = c_0 e^{\beta(\varepsilon_b^I - \varepsilon_{sol})}$$



- **Implications**:

In absence of ligand, this is a two-state system set by the energy difference between the on and off states and $\varepsilon_I - \varepsilon_A > 0$ which favors the on state.

For attractants, as L increases the receptor off state is favored, thus $K_I < K_A$.

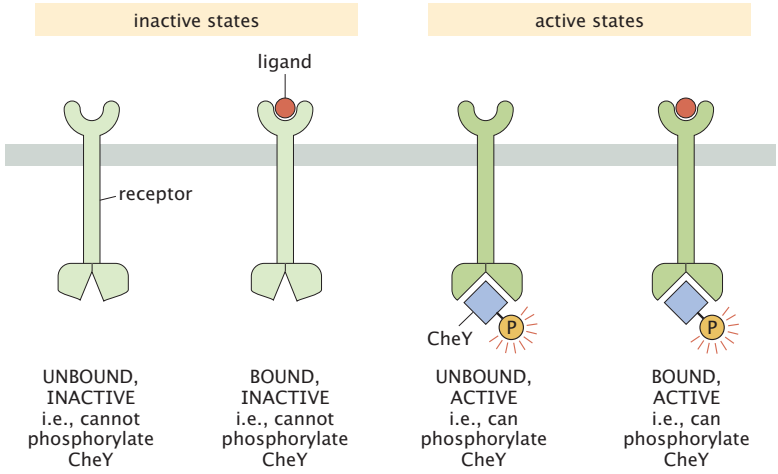
ε_I and ε_A can be regulated by covalent modifications of the receptor (methylation). The more a receptor is methylated, the more it is in the on state

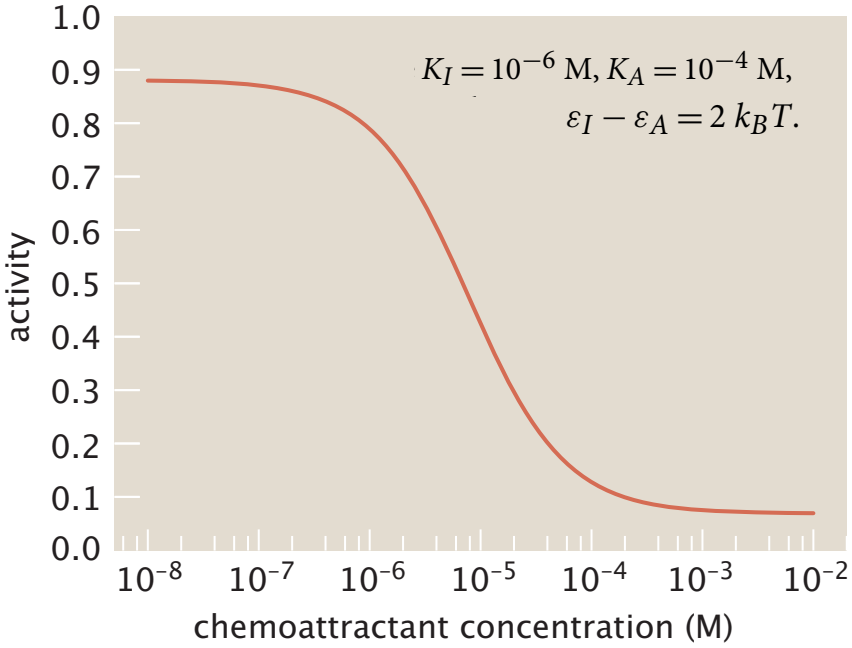
R. Phillips, The Molecular Switch: signaling and allostery. *Princeton Univ. Press*. 2020

Two-state allosteric model of chemotaxis

P_{active} probability that the receptor is active

$$p_{active} = \frac{1}{1 + e^{-\beta(\varepsilon_I - \varepsilon_A)} \dfrac{1 + (c/K_I)}{1 + (c/K_A)}}.$$





R. Phillips, The Molecular Switch: signaling and allostery. *Princeton Univ. Press*. 2020

Two-state allosteric model of chemotaxis

Sensitivity - Gain

Amplification by cooperative signaling

- Two-state model with cooperativity

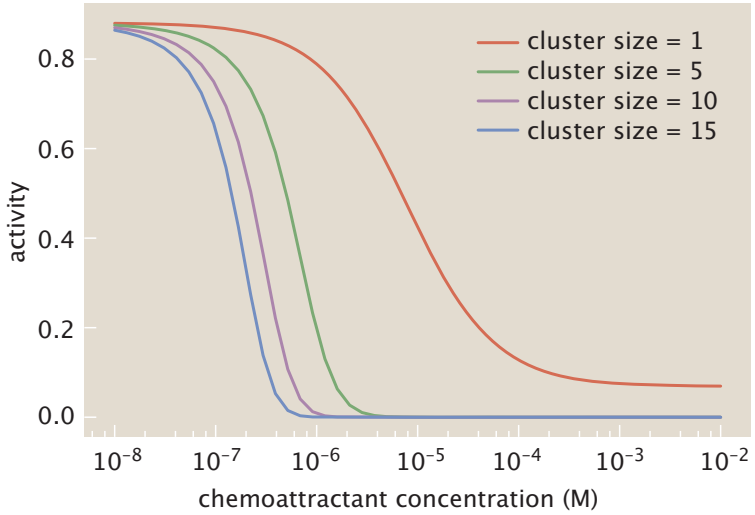
Binding of one receptor to its ligand affects the conformation of other adjacent receptors by allostery

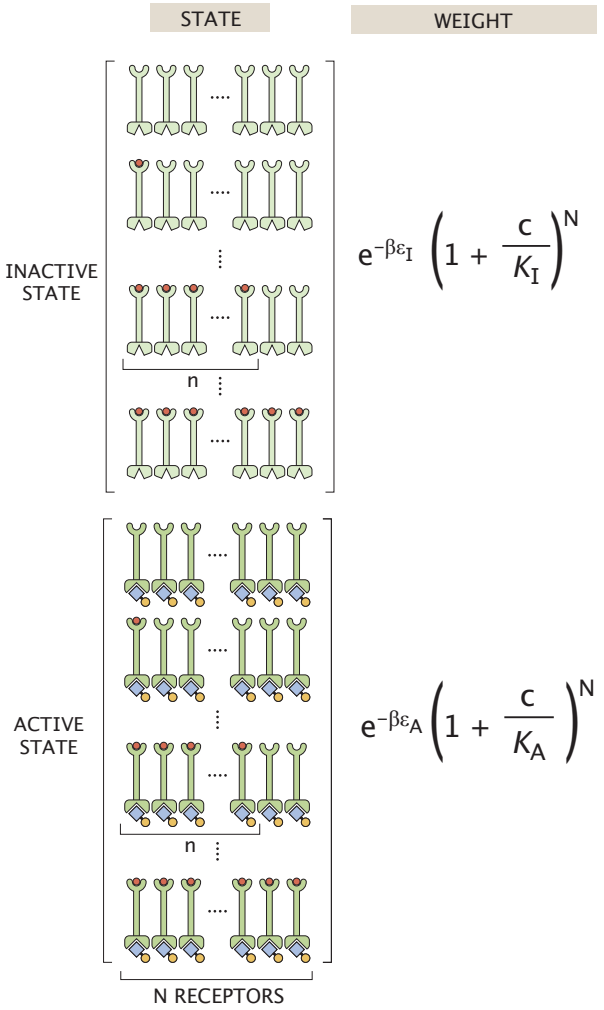
Cooperative interactions among receptors sharpens the response and increases its sensitivity

$$p_{active} = \frac{1}{1 + e^{-\beta(\varepsilon_I - \varepsilon_A)} \frac{\left(1 + (c/K_I)\right)^N}{\left(1 + (c/K_A)\right)^N}},$$

Sensitivity: fractional change in kinase activity/ fractional change in chemoattractant concentration

$$\text{sensitivity} = \frac{\frac{\Delta p}{p}}{\frac{\Delta c}{c}} = \frac{c}{p}\frac{dp}{dc} = \frac{d \ln p_{active}}{d \ln c}.$$

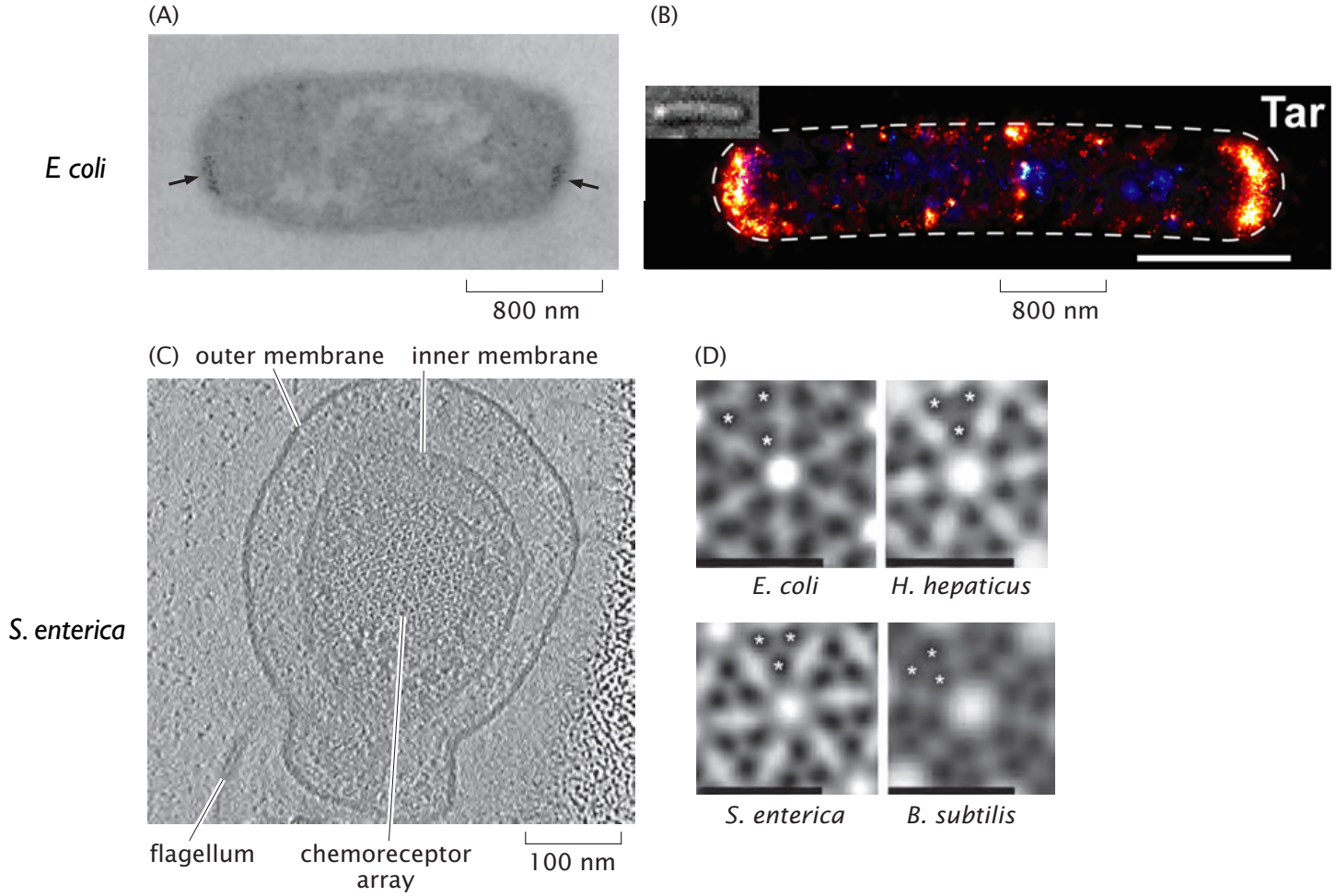


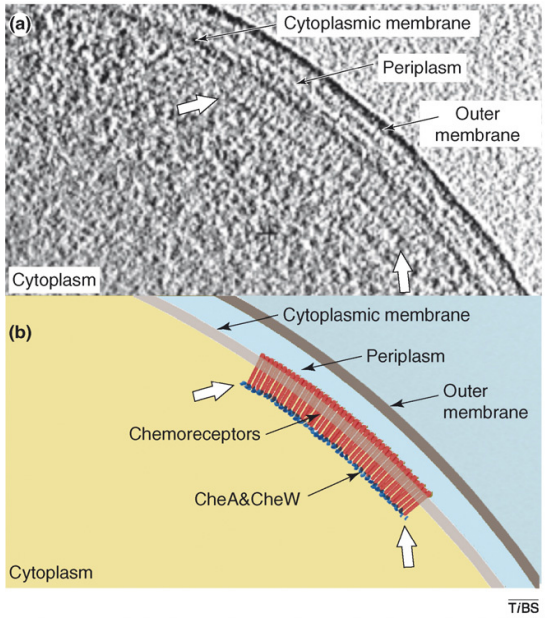


R. Phillips, The Molecular Switch: signaling and allostery. *Princeton Univ. Press*. 2020

Chemotaxis: sensitivity - gain

Amplification by cooperative signaling





J. R. Maddock and L. Shapiro, *Science* 259:1717, (1993)

Zhang, P. et al. *Proc. Natl. Acad. Sci.*. 104, 3777–3781 (2007)

D. Greenfield et al., *PLoS Biol*. 7:e1000137, (2009)

Briegel et al., *Proc. Natl. Acad. Sci*. 109:3766, (2012)

R. Phillips, J. Kondev, J. Thériot and H. Garcia. *Physical Biology of the Cell*. *Garland* 2012

Chemotaxis: sensitivity - gain

Amplification by cooperative signaling

- **The gain is extremely large:**

A step change in concentration of aspartate that increases the occupancy of the receptors by **1 molecule (1 part in 600, or 0.0016,** assuming 600 copies of the receptor Tar per cell) transiently increases the rotational bias by ≈0.1.

A ramp that increases the receptor occupancy by as little as 1 molecule/second leads to a steady state increase in bias pf a similar magnitude (0.1).

This corresponds to a change in run length by a factor of ≈3.

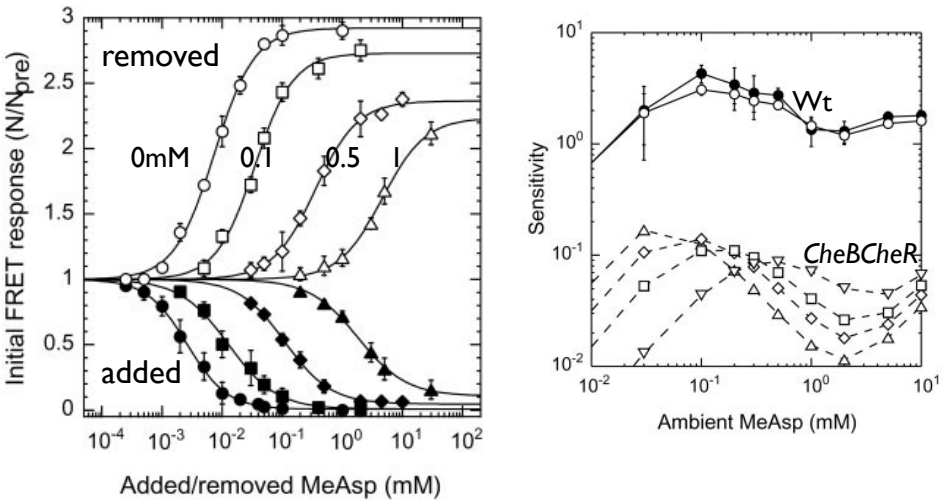
J. Segall, SM Block and & HC. Berg, *Proc. Natl Acad. Sci. USA* 83, 8987-8991 (1986).

Sensitivity: ratio of input change (ligand) to output change (CheYP measured by FRET)

$$\text{sensitivity} = \frac{\frac{\Delta p}{p}}{\frac{\Delta c}{c}} = \frac{c}{p}\frac{dp}{dc} = \frac{d \ln p_{active}}{d \ln c}.$$

- **Changes in Receptor occupancy up chemical gradients:**

« The changes in receptor occupancy encountered by bacteria swimming in spatial gradients (e.g., near the mouth of a capillary tube in the capillary assay) are very small. For example, in the tracking experiments, cells about 0.6 mm from the tip of a capillary tube containing 1 mM aspartate moved in a gradient of steepness 0.02 μM/μm at a mean concentration of about 8 μM. A 10μm run straight up such a gradient would change the concentration from 8 to 8.2 μM, i.e., by 2.5%. Assuming K_D values for aspartate of 7.1 μM and 62 mM, this step gives a **fractional change in receptor occupancy of about 0.003**. »



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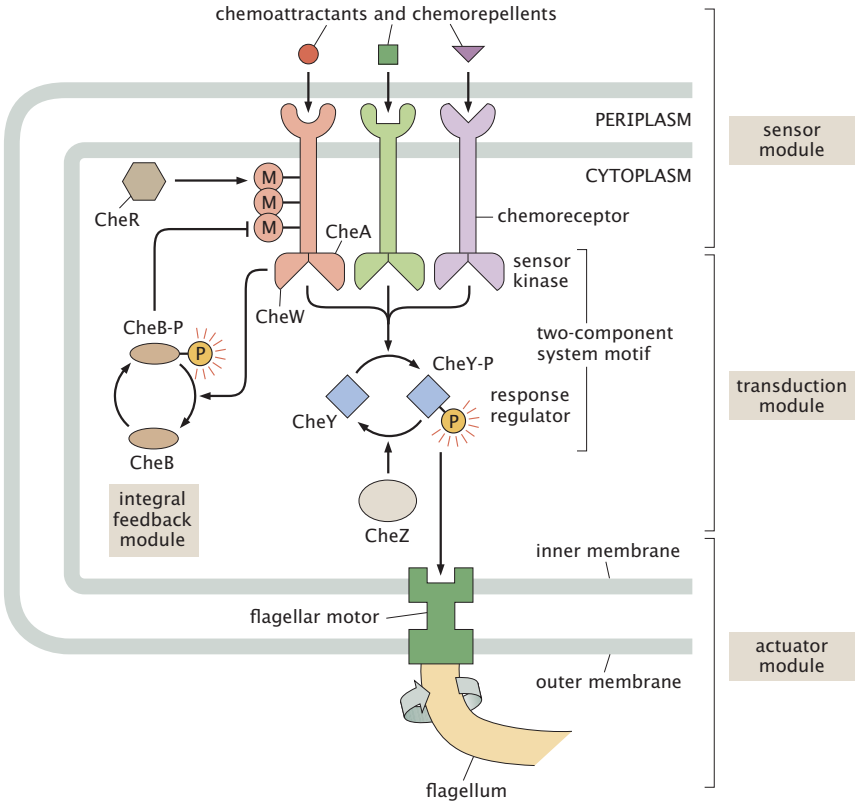
Chemoreceptor physiology

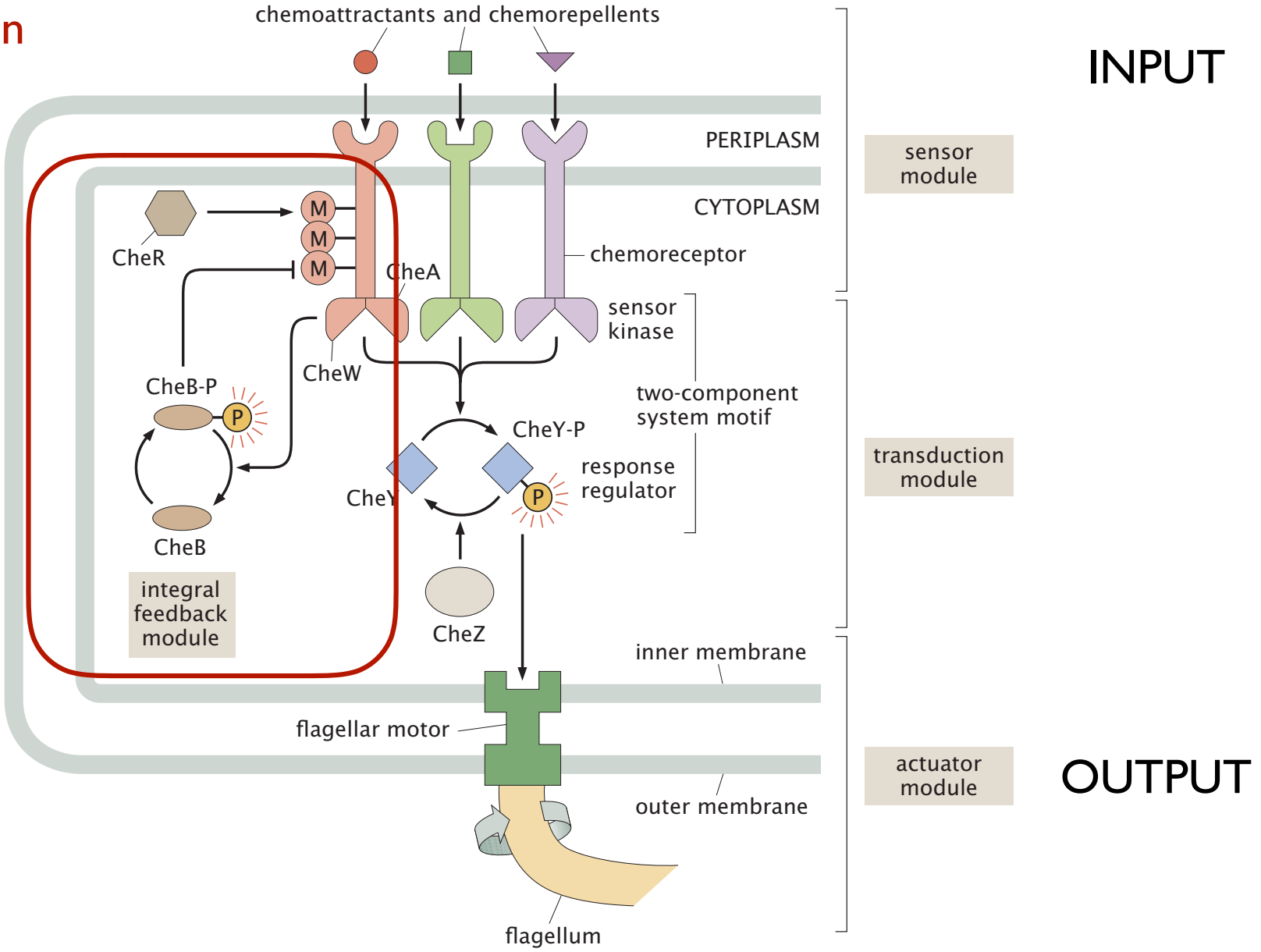
Key properties of chemotactic network



- Sensitivity - Gain : output/input ratio
- Adaptation: reset after input
- High amplitude range

Chemoreceptor physiology

- Adaptation



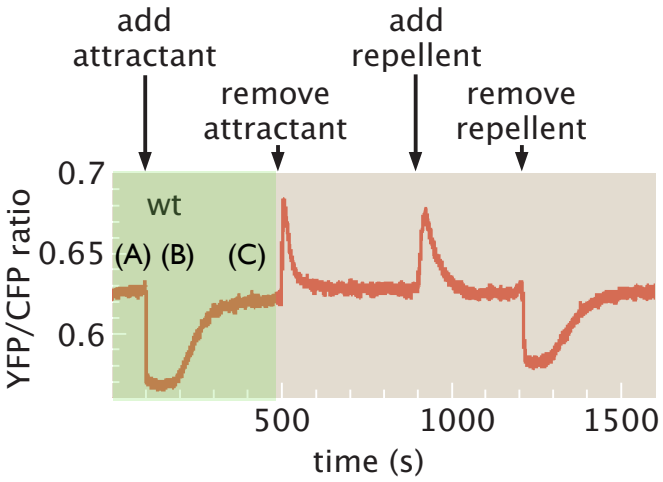
R. Phillips, The Molecular Switch: signaling and allostery. *Princeton Univ. Press*. 2020

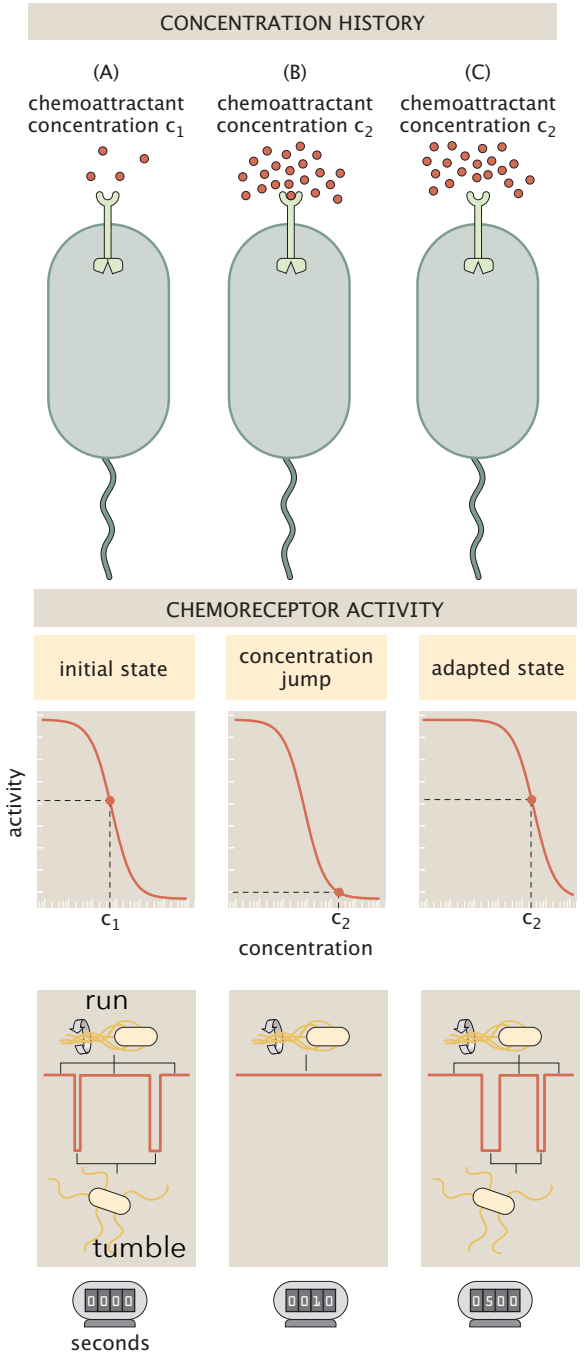
Adaptation

The evidence/principle

- (A)-(B) Cells respond to an increased concentration of attractant (c_2) by lowering activity
  (reduced FRET reflects reduced CheY-P concentration)

- (B)-(C) Then cells restore/reset their activity: they adapt to the new stable concentration c_2





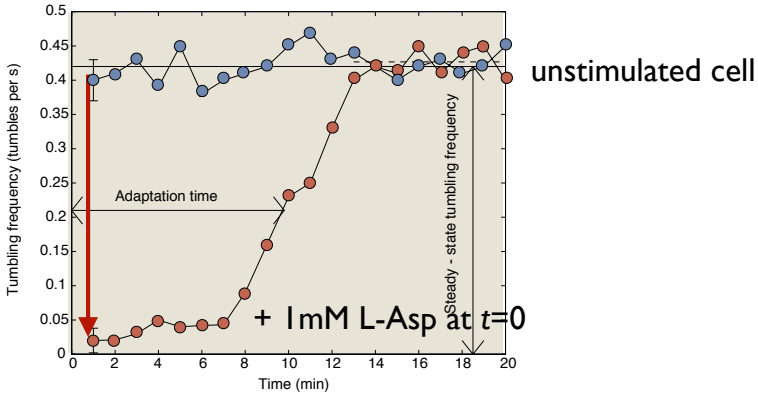
Sourjik, V. & Berg, H. C. *Proc. Natl Acad. Sci. USA* 99, 123–127 (2002).

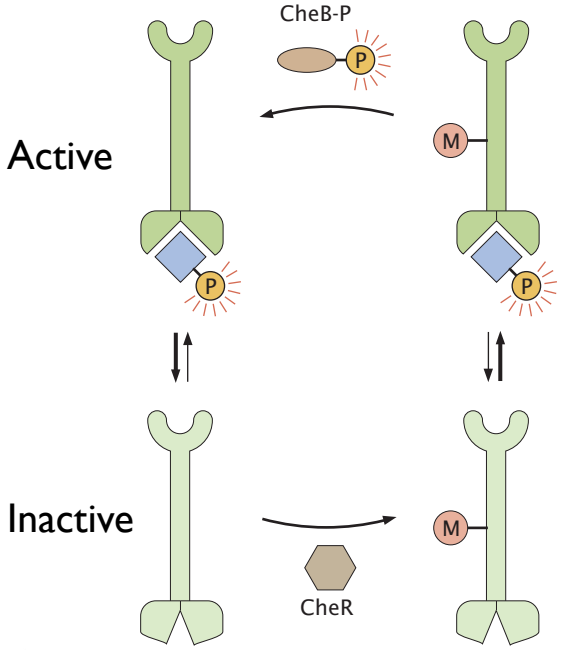
R. Phillips, The Molecular Switch. *Princeton Univ. Press*. 2020

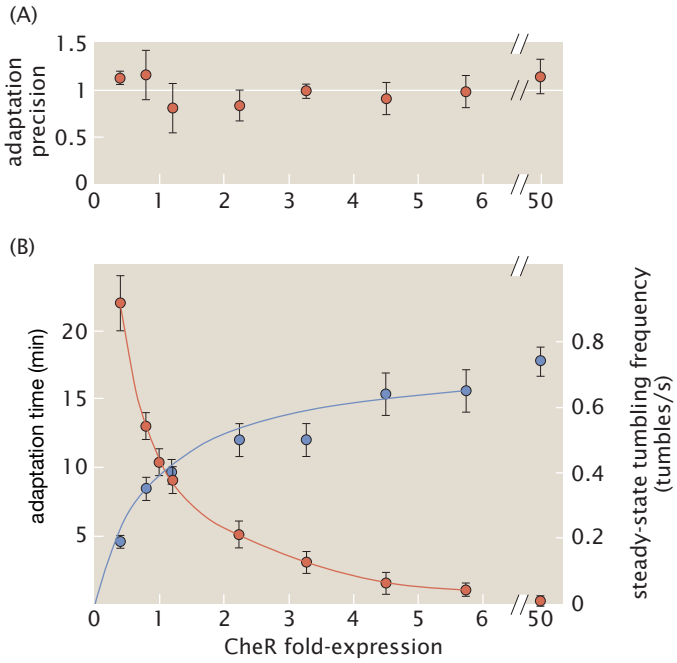
Adaptation

The evidence/principle

- Adaptation requires reversible methylation of the chemoreceptors by methylation enzyme CheR and the demethylase CheB
- CheR adds methyl when the receptor is inactive
- CheB is activated (-P) by the active receptor (bound ligand), and removes methyl.
- Adaptation time decreases as CheR concentration increases







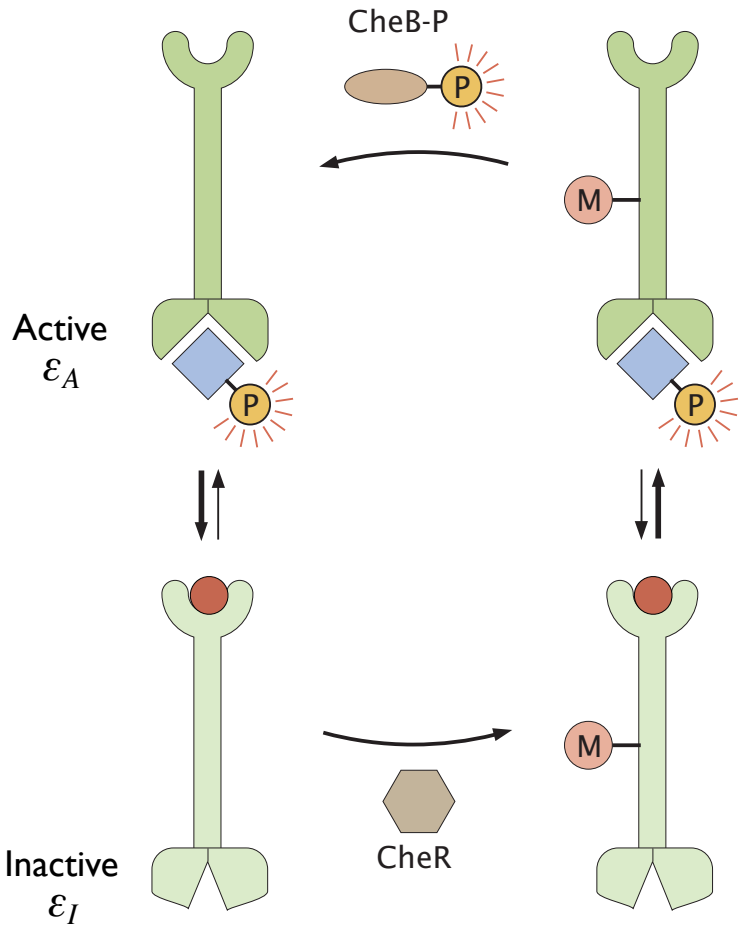
precision is the ratio between the steady-state tumbling frequency of unstimulated and stimulated cells

Alon, U, Surette, MG, Barkai, N, & Leibler, S *Nature* 397, 168. (1999)

Adaptation

The mechanism

- Core of adaptation: Resetting
- **Methylation is updated by receptor activity (and ligand binding):** The demethylase CheB is regulated by phosphorylation by CheA, and thus by ligand binding to the receptor.
- Without ligand, the receptor is on, CheA is active and so CheB is more active as a demethylase, and will convert the receptor into the off state. The response is damped.
- Conversely, with attractant, the receptor is off, CheB is less active, methylation accumulates, and the receptor becomes on.

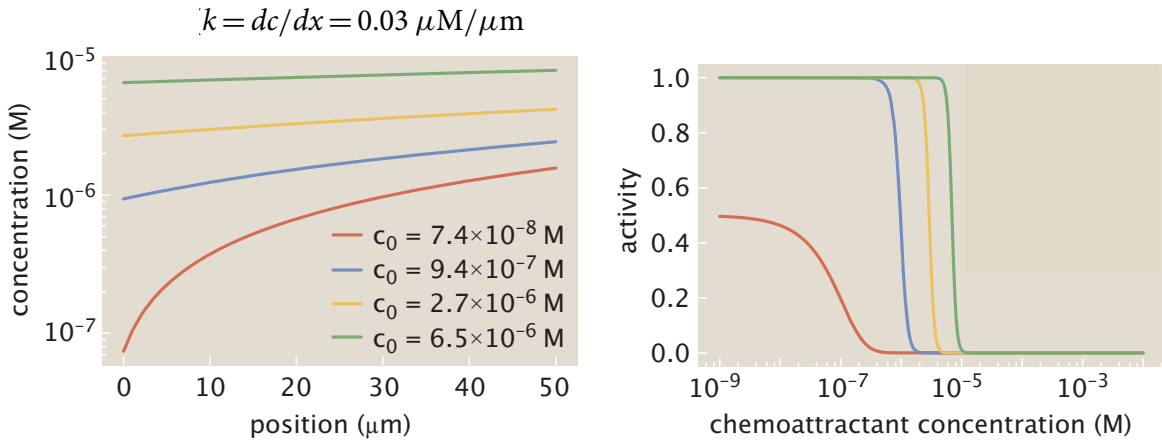


R. Phillips, The Molecular Switch. *Princeton Univ. Press*. 2020

Adaptation

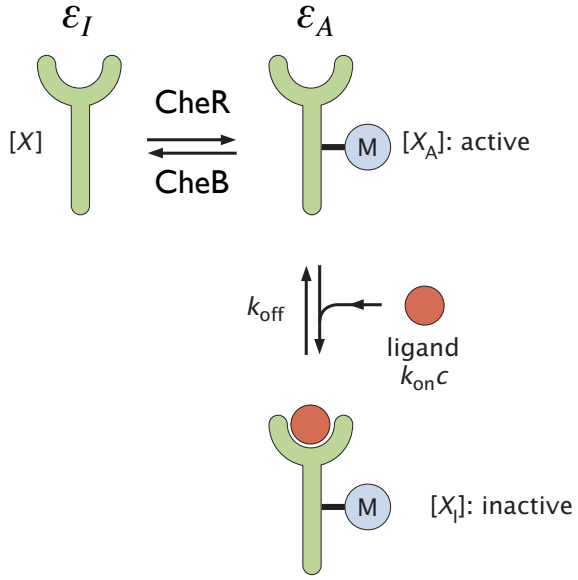
The mechanism

- Concentration gradient: $c(x) = c_0 + kx,$
- In the same concentration gradient cells have different input-output function when the background concentration is different.



- This can be explained in terms of a different energy difference $\Delta\varepsilon = \varepsilon_I - \varepsilon_A$ between the active and inactive states of the receptor in absence of ligand.

$$p_{active} = \frac{1}{1 + e^{-\beta\ \Delta\varepsilon}\ \left(\frac{1 + (c/K_I)}{1 + (c/K_A)}\right)}.$$



R. Phillips, The Molecular Switch. *Princeton Univ. Press*. 2020

Chemoreceptor physiology

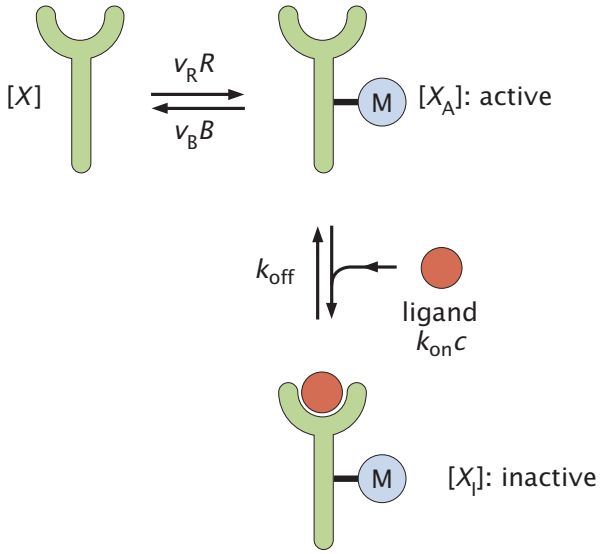
Mechanism of Adaptation

- **Two state model of precise adaptation:**

Methylation of receptor reflects ratio of CheB and CheR

Negative feedback: Demethylation mediated by CheB only happens in the active state of the receptor. As a result, if the ligand concentration changes, the number of active receptors X_A will change, and so will the ratio of CheB and CheR. Therefore, the number of methylated active receptor will change in opposite direction.

For instance, if chemoattractant increases, inactive receptors go up, but meanwhile, CheR will win over CheB which is less active, so overtime more active receptor X_A.



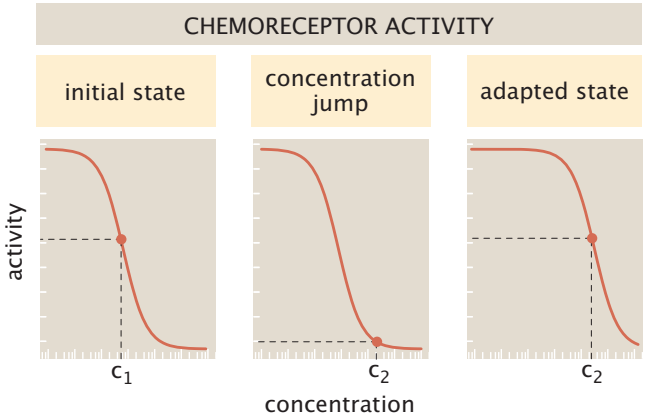
R. Phillips, J. Kondev, J. Thériot and H. Garcia. *Physical Biology of the Cell*. *Garland* 2012

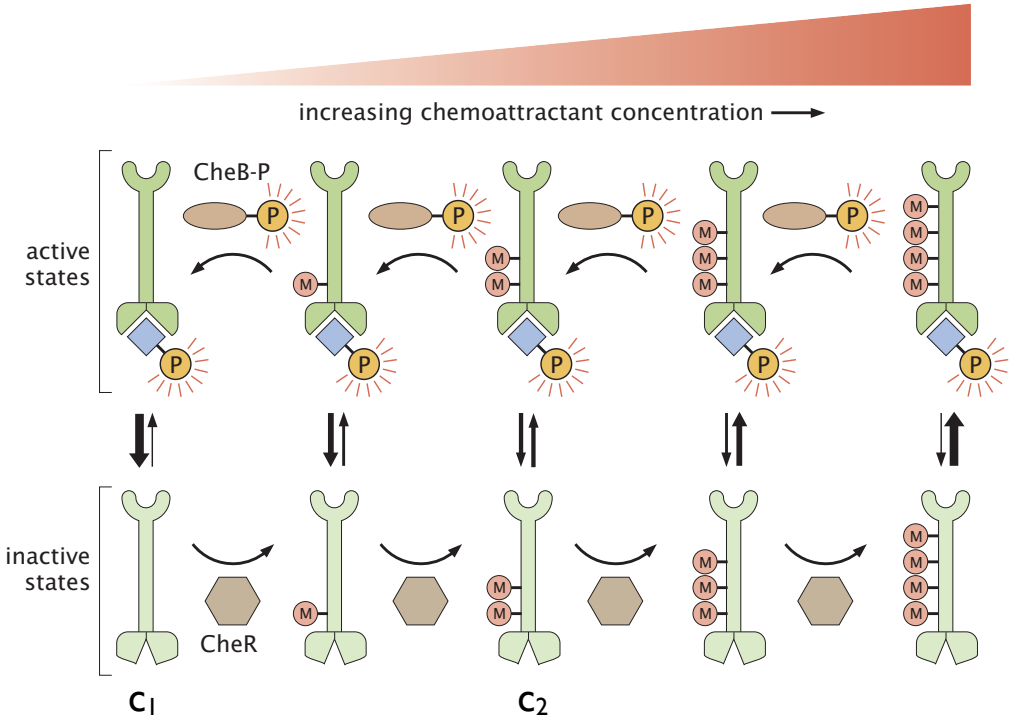
Adaptation

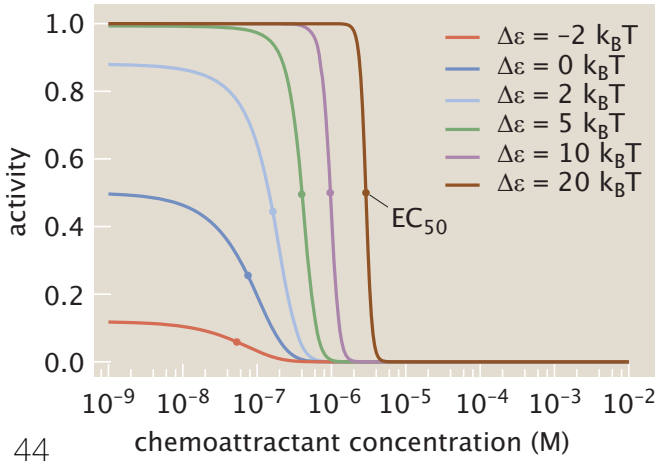
The mechanism

- Adaptation entails different methylation status (up to 4) regulated by CheR and CheB

  Each methylation status corresponds to different values of $\Delta\varepsilon$ and thus different EC50, though K_I and K_A are unchanged







$K_I = 10^{-6}$ M

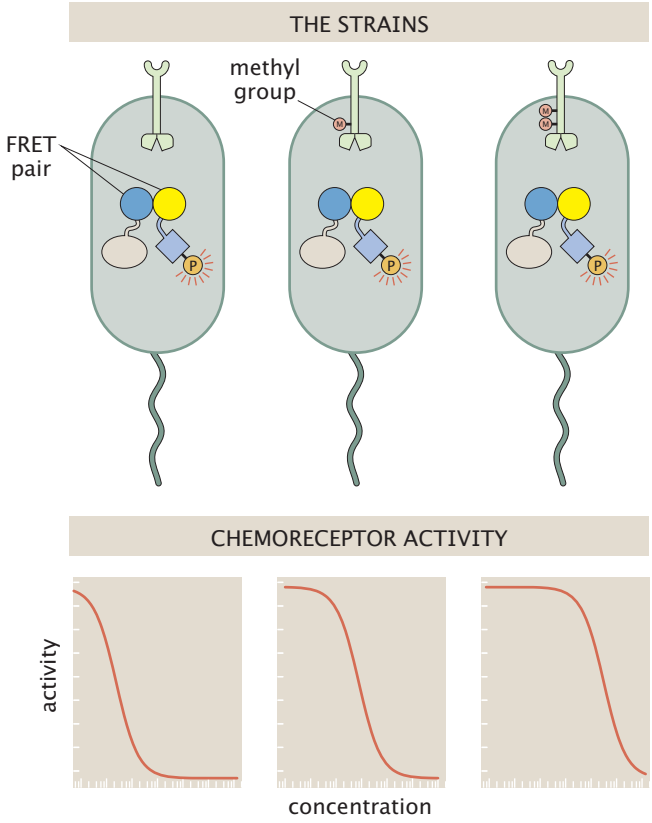
$K_A = 10^{-4}$ M

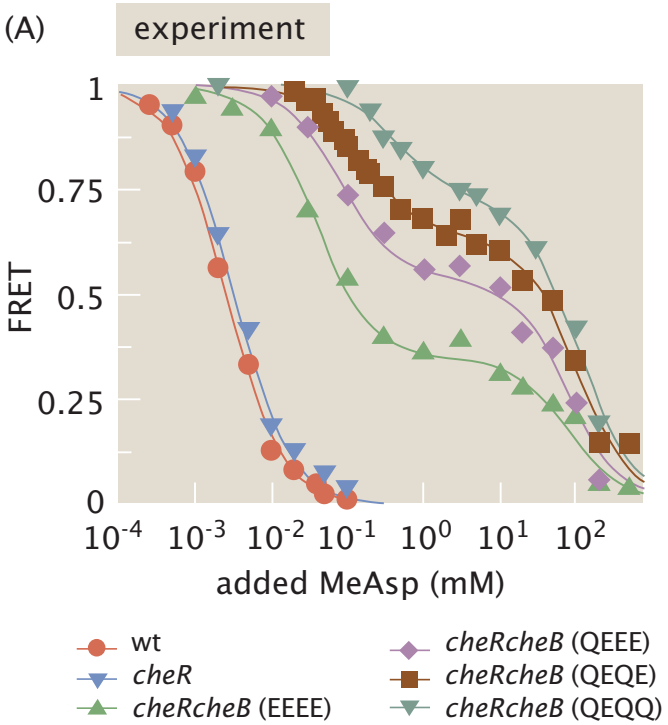
R. Phillips, The Molecular Switch. *Princeton Univ. Press*. 2020

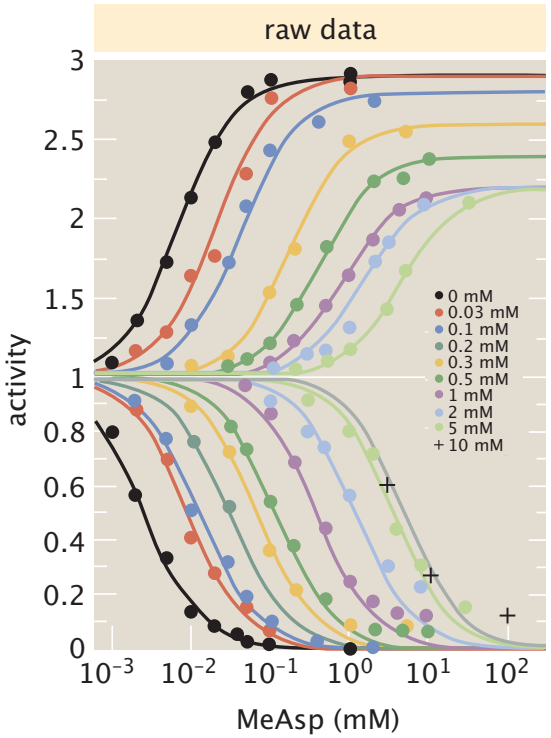
Adaptation

The mechanism

- Increased methylation increases the EC50.
- The sweet spot of the input-output response is shifted to higher concentration of the ligand
- This enables response to a very large range of ligand concentration in a chemotactic gradient
- Indeed, a higher ambiant concentration of the ligand increases the EC50 in stepwise increase in ligand concentration







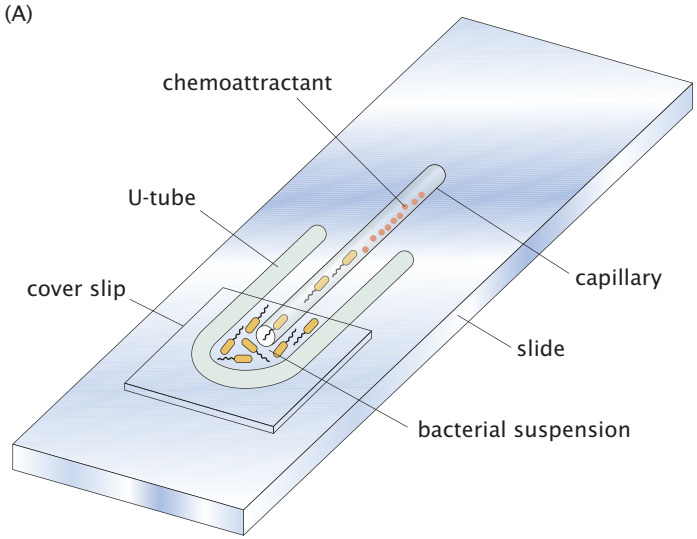
R. Phillips, The Molecular Switch. *Princeton Univ. Press*. 2020

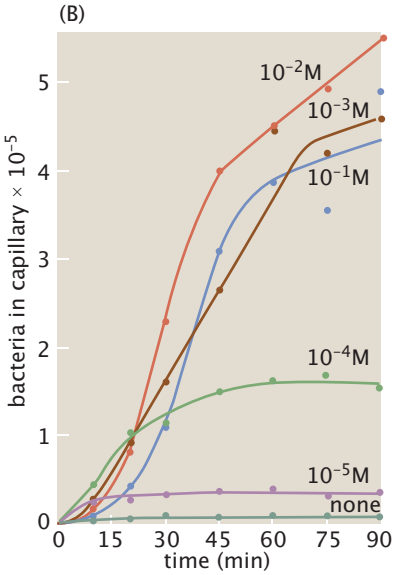
Sourjik, V. & Berg, H. C. *Proc. Natl Acad. Sci. USA* 99, 123–127 (2002).

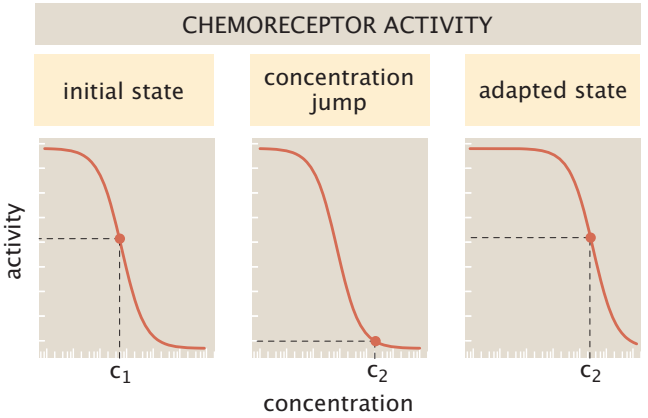
Keymer, J et al. & Wingreen, N. *Proc. Natl Acad. Sci. USA* 103, 1786-1791 (2006)

Chemoreceptor physiology

Adaptation allows cells to sense over a wide range of concentration



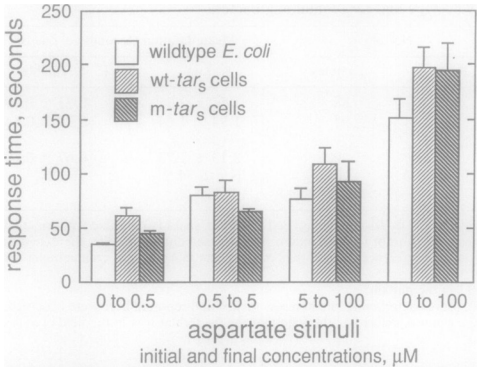


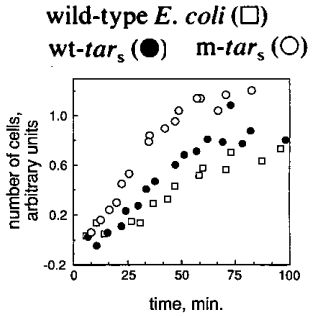


Adaptation is required for chemotaxis

- Cells that can adapt but have a different tumbling frequency from wild type are chemotactic (they can go up a gradient of attractant)

CW bias in wildtype *E. coli* 37%,
E. Coli expressing wt or mutant *Salmonella* Tar receptor wt Tar_S 56%, and mutant mTar_S 18%
Yet, all cells show similar chemotactic response: response or adaptation time

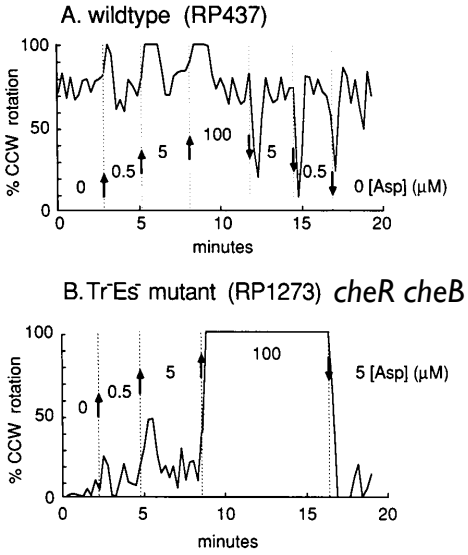




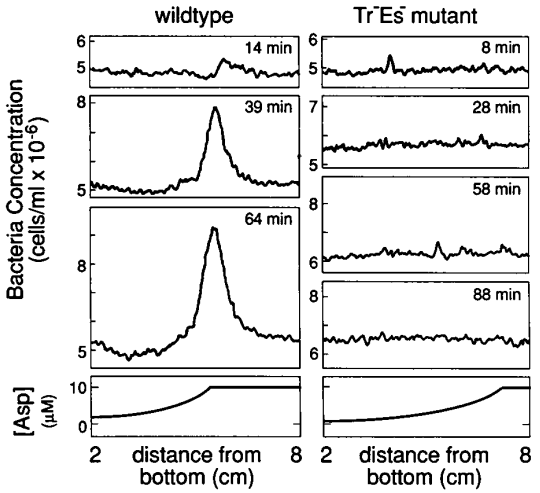
response to gradient

Weis, R. M. & Koshland, D. E. *J. Bacteriol*. 172, 1099±1105 (1990).

- However, cells that have a normal tumbling frequency but which cannot adapt are defective in chemotaxis



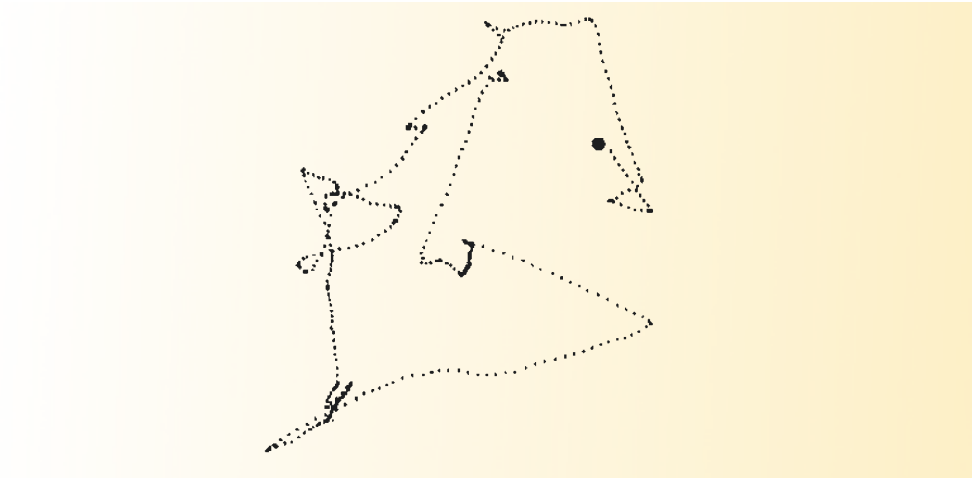
defective adaptation



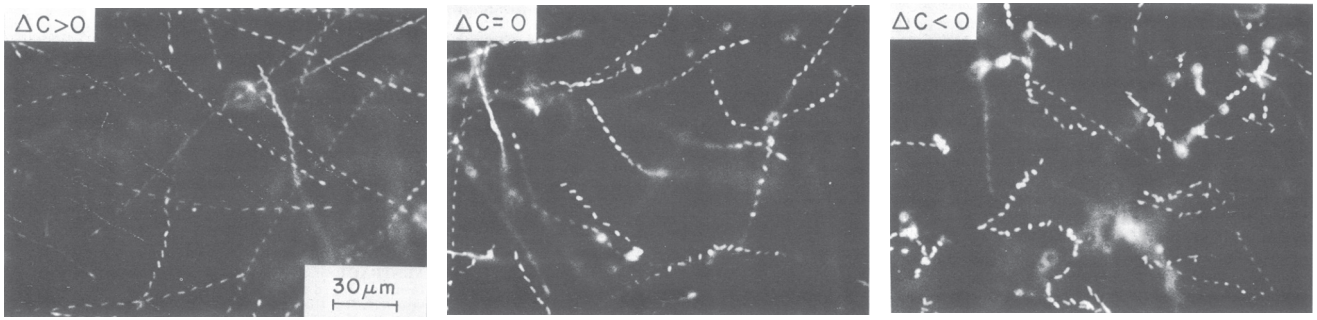
Defective chemotaxis in gradient

Weis, R. M. & Koshland, D. E. *Proc. Natl Acad. Sci. USA* 86, 83±87 (1988)

Chemotaxis entails detection of a temporal gradient



- Bacteria detect a temporal change in concentration of chemoattractant
- As they navigate in space, they detect in time different concentrations
- This requires comparison of 2 measurements and memory



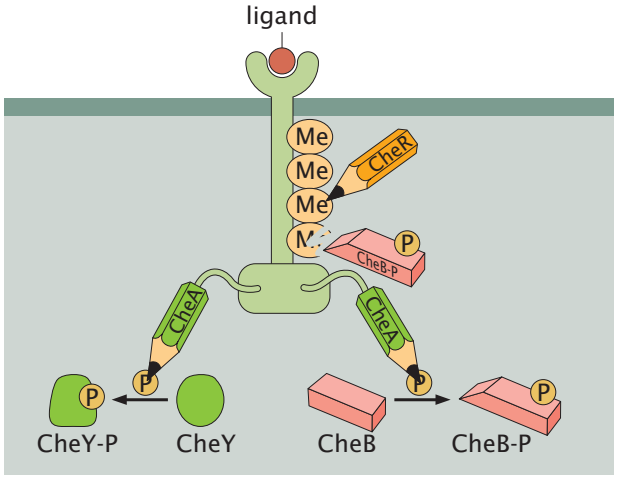
Salmonella typhimurium R. Macnab. D.E. Koshland. *PNAS*. 69:2509-2512 (1972)

How is adaptation required for chemotaxis?

- Cells have a built-in short term memory to compare present and recent past and thereby read the concentration gradient

- Methylation and demethylation take a few seconds and thus reflect receptor activity a few seconds ago (« memory »).
- Receptor occupancy by ligand influences the current activity state (which takes a fraction of a second). By comparing the activity state of the cell (CheA) and methylation, the cell can compute how signal evolved in a few seconds, whether it increased, or decreased.

Fast (<<1s)

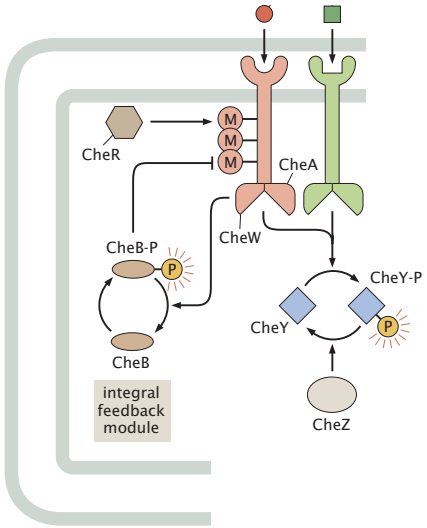


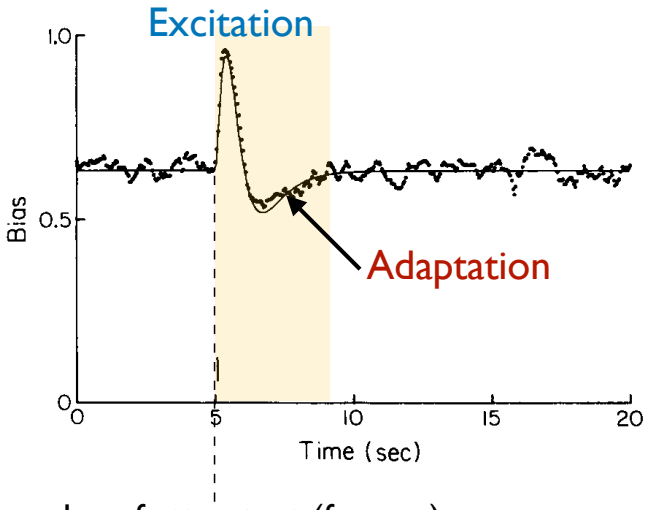
Longer (2-3 s)

How is adaptation required for chemotaxis?

- Cell response is integrated over few seconds: response to very short pulse (ms), lasts about 4 seconds, the signal persists after the ligand is no longer present at the cell surface (it diffuses away within a fraction of a second).
- The response is biphasic (2 lobes): Cells increase their CCW bias, ie. they run for about 1s, then, reduce it and undershoot below the steady state value, and catch up. In other words, cells run smoothly for 1s (≈30µm distance), then tumble for 3s and catch up.

This indicates that cells perceive changes in concentration during this time interval
(signature of impulse: on/off switch)





Impulse of attractant (few ms)

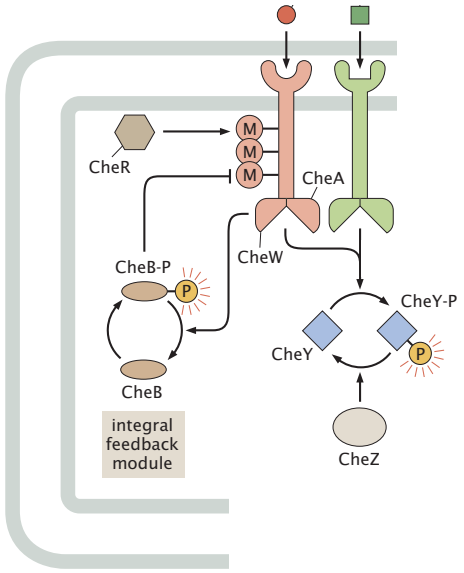
Chemotactic network response:

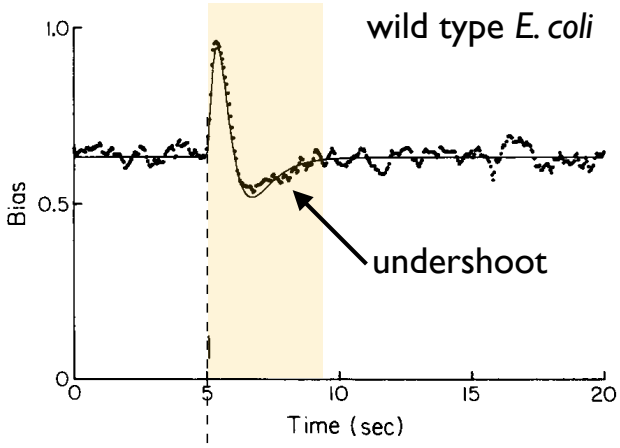
First few ms: the attractant binds to receptors, triggers a signal that raises the flagellar bias and stimulates receptor methylation. The concentration of attractant rapidly decreases (ms); the attractant leaves the receptors and diffuses away, but the signal persists.

The imbalance between receptor occupancy (off state) and methylation (which pushes towards on state) eventually causes the signal to fall below its prestimulus value. This lowers the bias below its prestimulus level and stimulates demethylation. Finally, as methylation returns to its original level, the signal follows, and the bias returns to its prestimulus level.

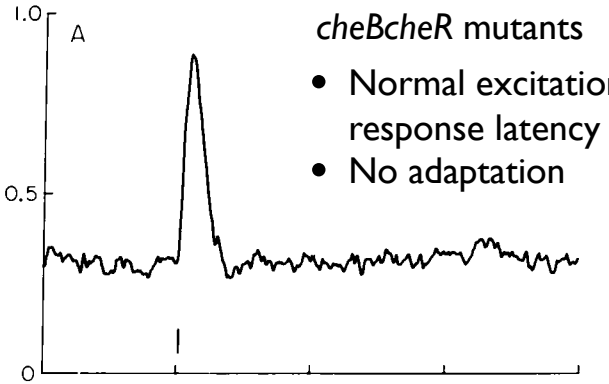
SM. Block, J. Segall and H. Berg. *Cell*,. 31, 215-226 (1982)
J. Segall, SM. Block and HC. Berg. *PNAS*. 83:8987-8991 (1986)

How is adaptation required for chemotaxis?



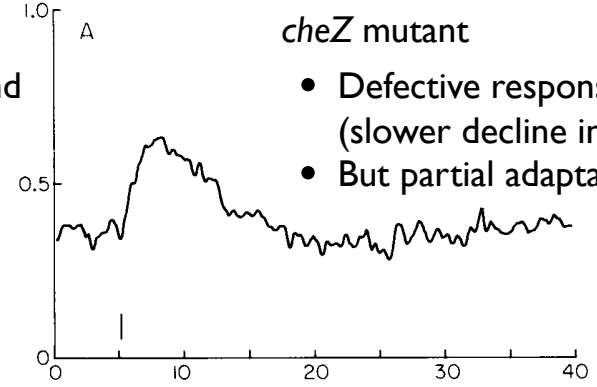


- Cells compare the response in first 1s (positive lobe), and next 3s (negative lobe).
- The comparison is a consequence of the adaptation mechanism
- Without adaptation, cells have no memory of recent past, and cannot read temporal gradient, hence cannot do chemotaxis.



cheBcheR mutants

- Normal excitation and response latency
- No adaptation

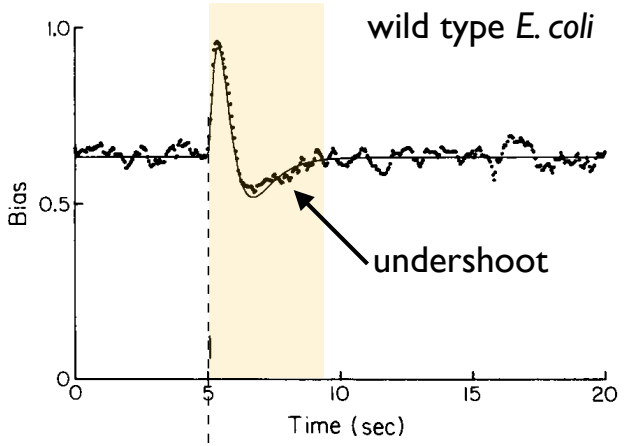


cheZ mutant

- Defective response latency (slower decline in activity)
- But partial adaptation

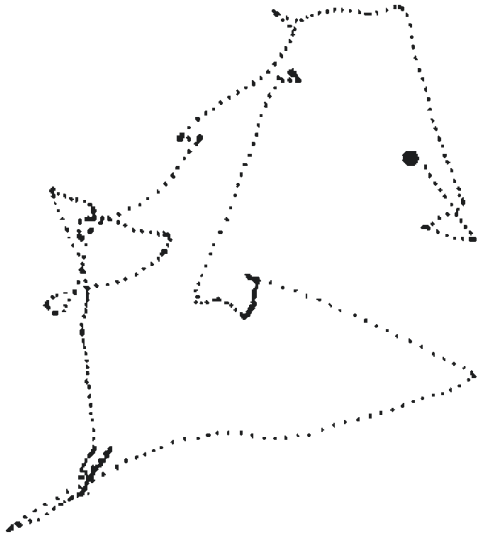
SM. Block, J. Segall and H. Berg. *Cell*,. 31, 215-226 (1982)
J. Segall, SM. Block and HC. Berg. *PNAS*. 83:8987-8991 (1986)

How is adaptation required for chemotaxis?



- Any signal fluctuation at higher frequency than this will not be sensed (low pass filter)
- Fluctuation on longer time scale will not be perceived either (high pass filter)
- **The chemotactic network operates as a band pass filter.**

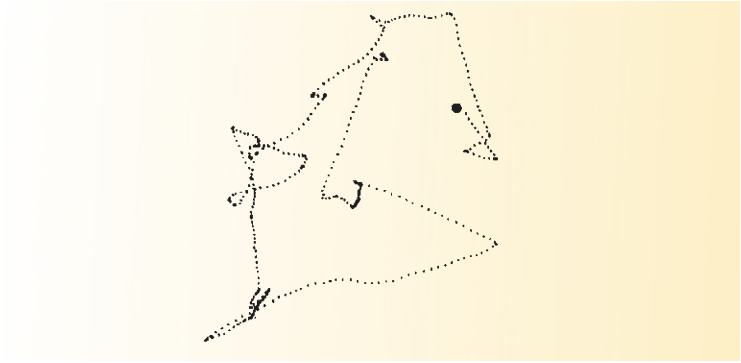
- Cells have an optimum pass frequency of 0.25Hz, which is 4s. With a succession of runs of about 1s, cells experience change in concentration in the range of 0.25Hz.
  So cells are optimized to read concentration differences in a gradient



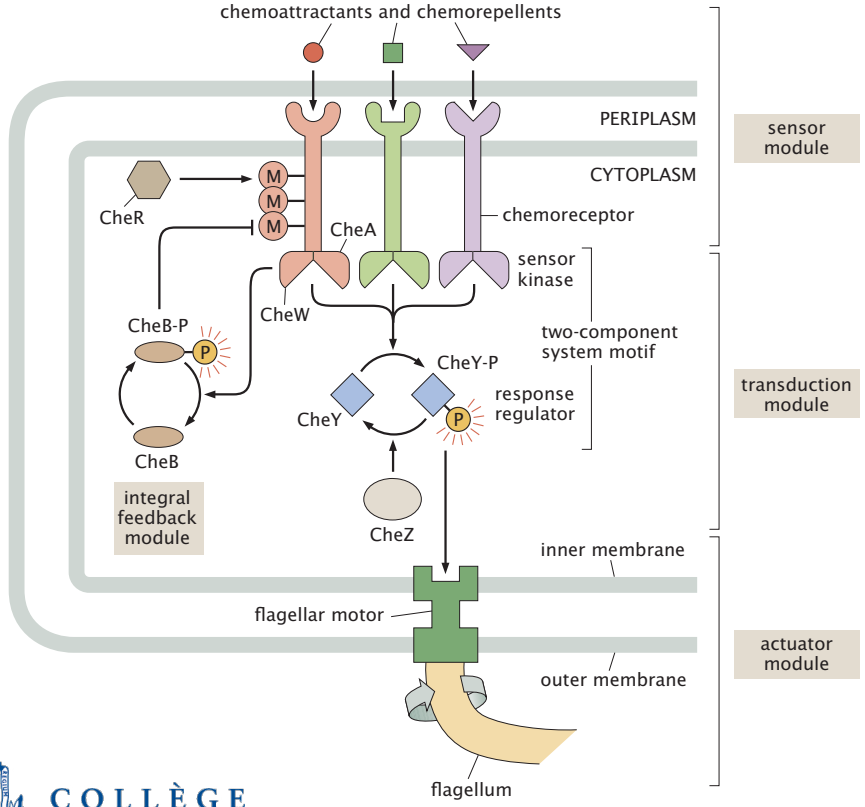
- Runs must be long enough to outrun diffusion (>1s) and ignore stochastic fluctuations
- Runs must be below 10s, the time interval over which rotational diffusion (≈30° in 1s) of *E. coli* is such that cells would lose information about where favorable concentrations are.
- A cell that integrates over such a long time (eg. CheZ mutant), namely a cell with excessive memory, will have information about the past that is no longer relevant to the current trajectory: the cell cannot be chemotactic.

SM. Block, J. Segall and H. Berg. *Cell*,. 31, 215-226 (1982)
J. Segall, SM. Block and HC. Berg. *PNAS*. 83:8987-8991 (1986)

Conclusions



- Biased random walk in a spatial gradient
- Temporal gradient sensing
- Memory



Key properties of chemotactic network

- Sensitivity - Gain : output/input ratio
- Adaptation: reset after input
- High amplitude range