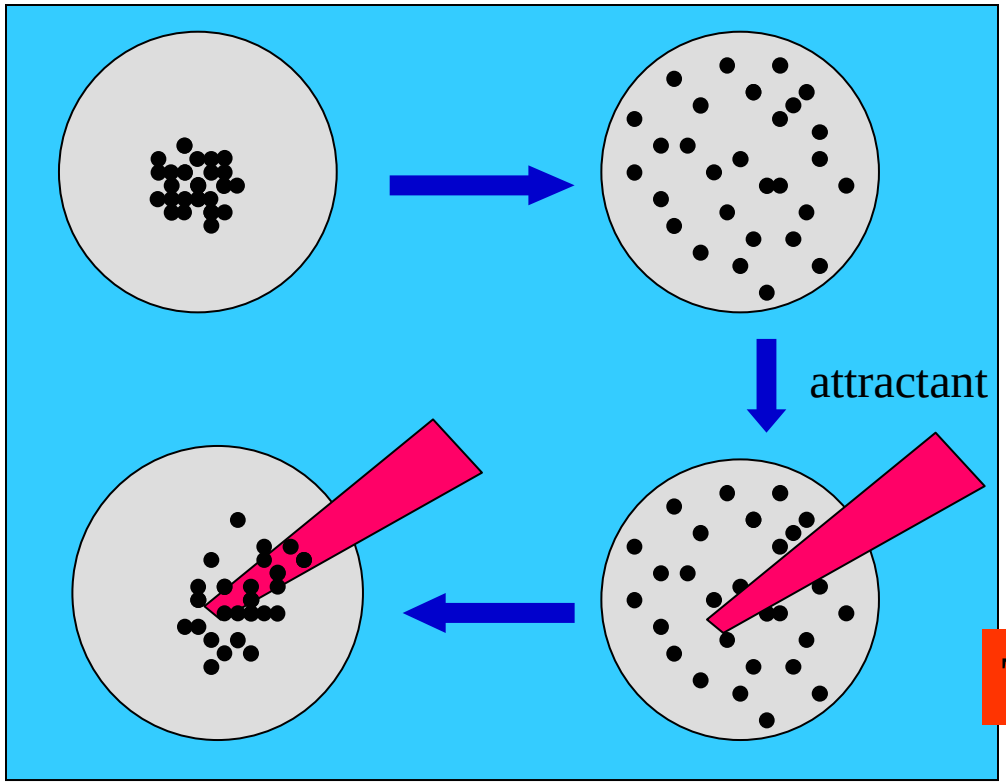
Bacterial Chemotaxis

Bacteria can be attracted/repelled by chemicals



Mechanism ?

"Chemoreceptors in bacteria."

Adler, 1969 "Science" – READ!

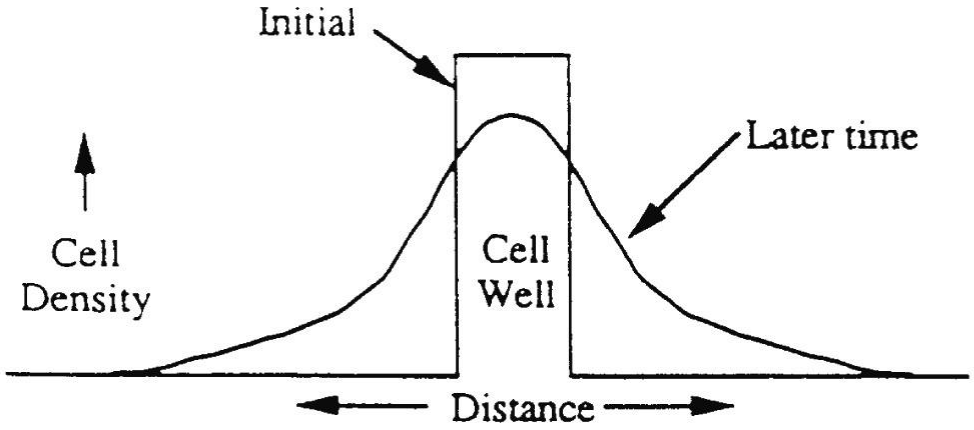
This is sensing, not metabolism

Based on genetic approach!!!
No molecules yet

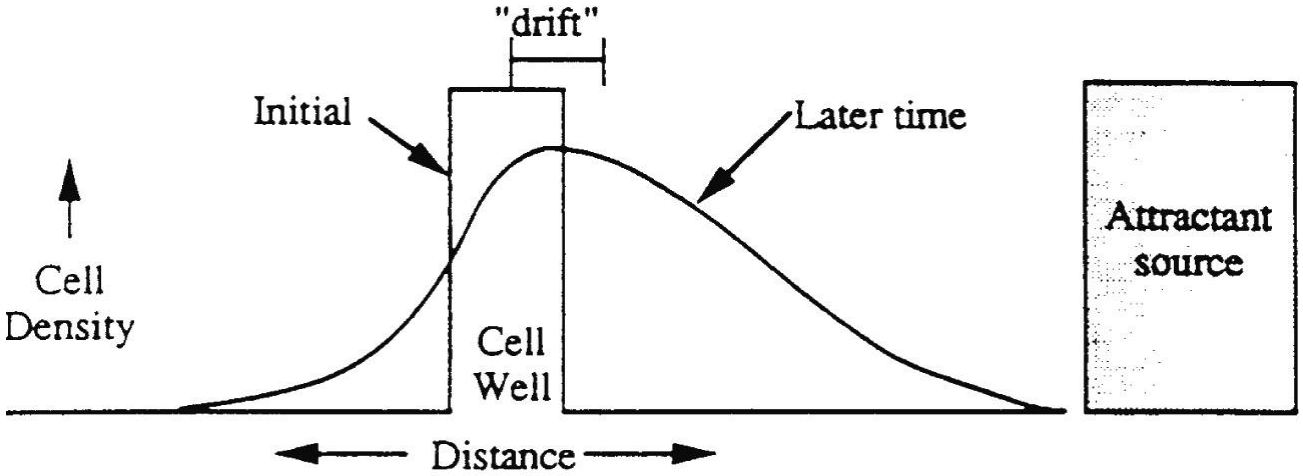
Macroscopic phenomenon:

flux of bacteria = F(gradient of chemicals)

Random Motility and Chemotaxis

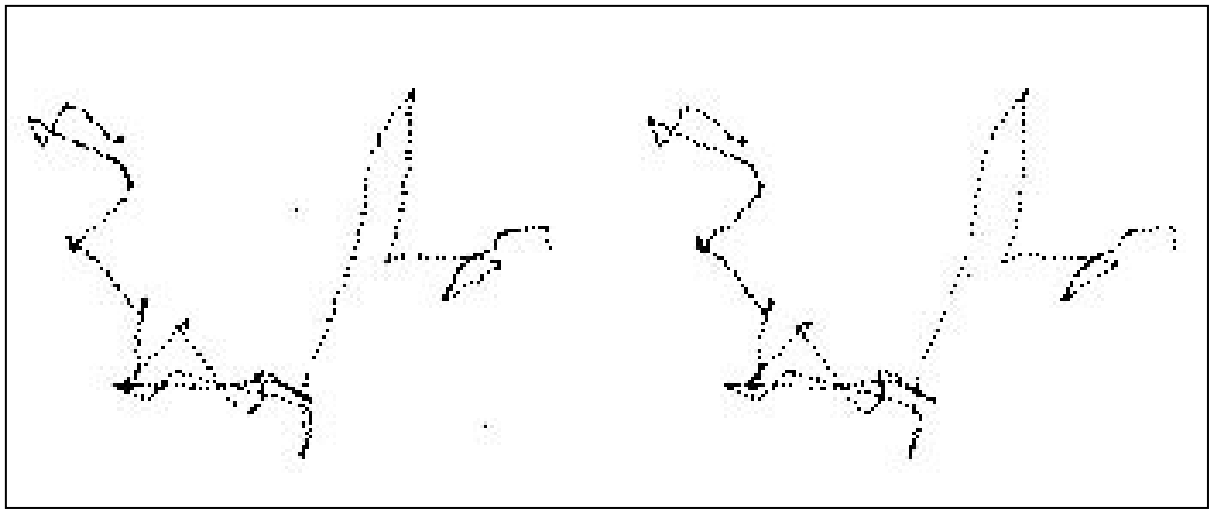


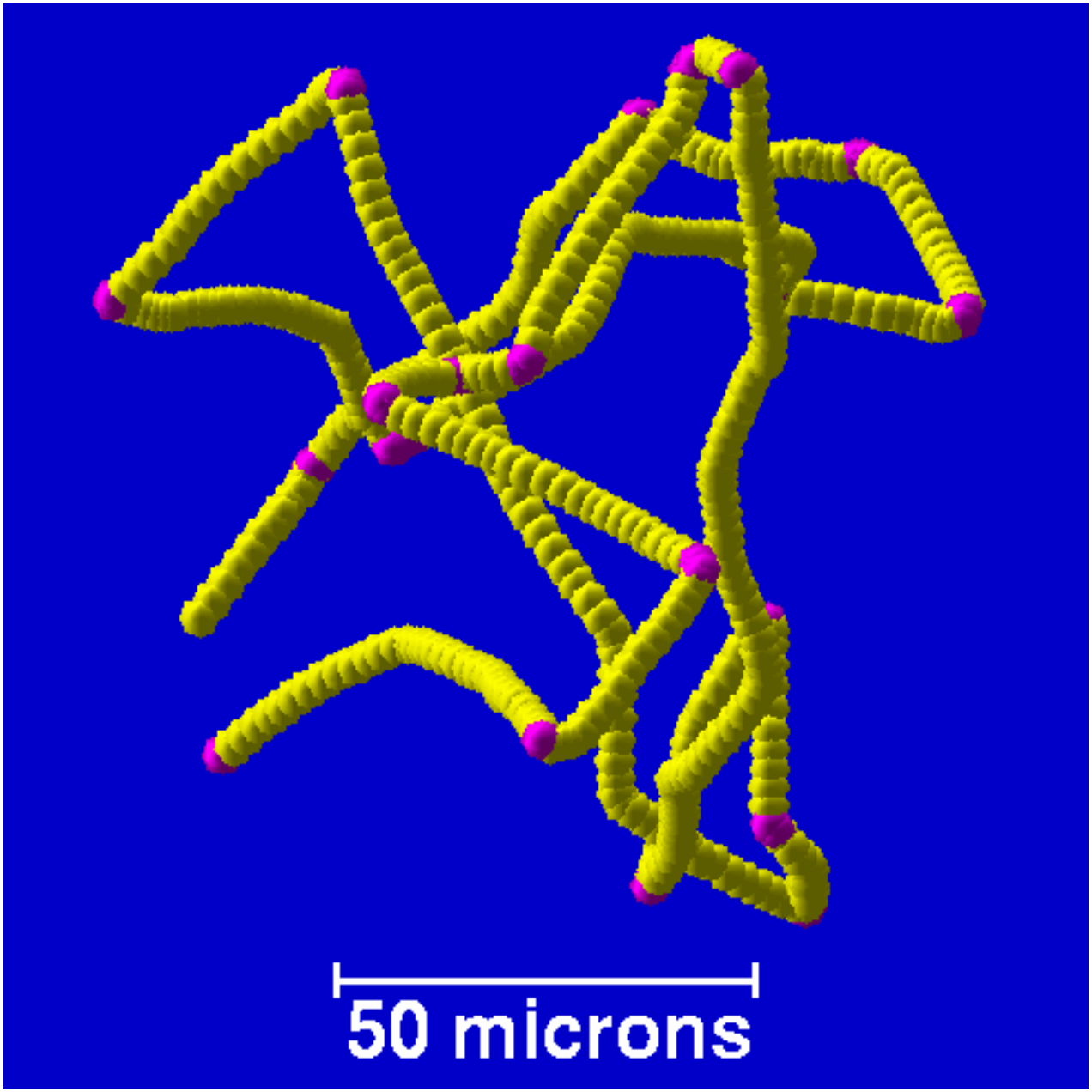
b. Migration in a chemical attractant gradient:
Random motility (μ), chemokinesis ($\frac{d\mu}{da}$) and chemotaxis (χ)



Trajectories

In the absence of chemical gradients, a swimming bacterium executes a three-dimensional random walk consisting of **runs** of swimming in a straight line punctuated by tumbles

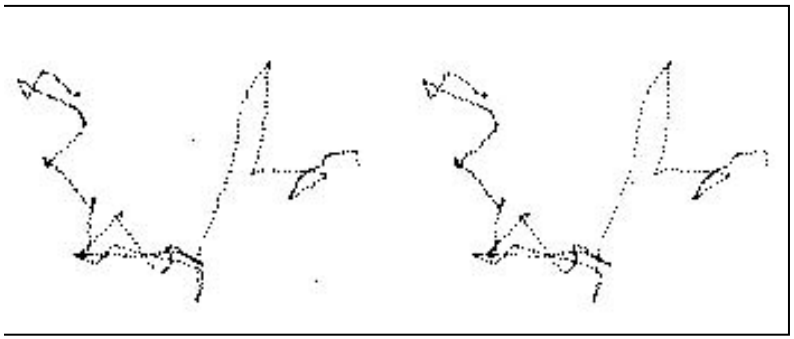



50 microns

From Trajectories to Microscopic Parameters of Cell Migration

(Velocity Jump Process)

Berg and Brown, 1972

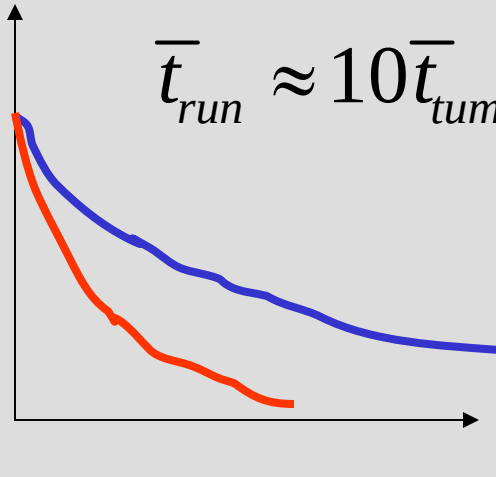


$P\{T_{run} > t\}$

or

$P\{T_{tumble} > t\}$

$\bar{t}_{run} \approx 10\bar{t}_{tumble}$

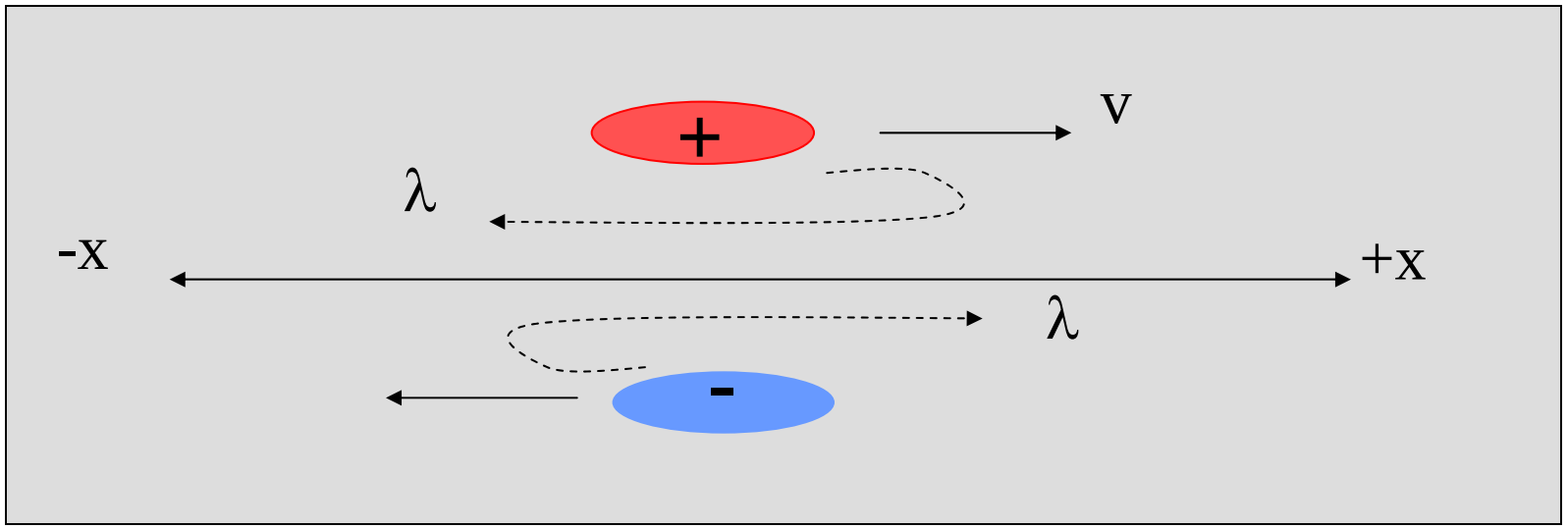


1. Runs punctuated by tumbles
2. Both runs and tumbles are exponentially distributed
3. Runs are longer than tumbles
4. Constant velocity

MODEL: instantaneous tumbles (neglect tumble time)

MODEL: instantaneous tumbles (neglect tumble time)

Velocity Jump Process



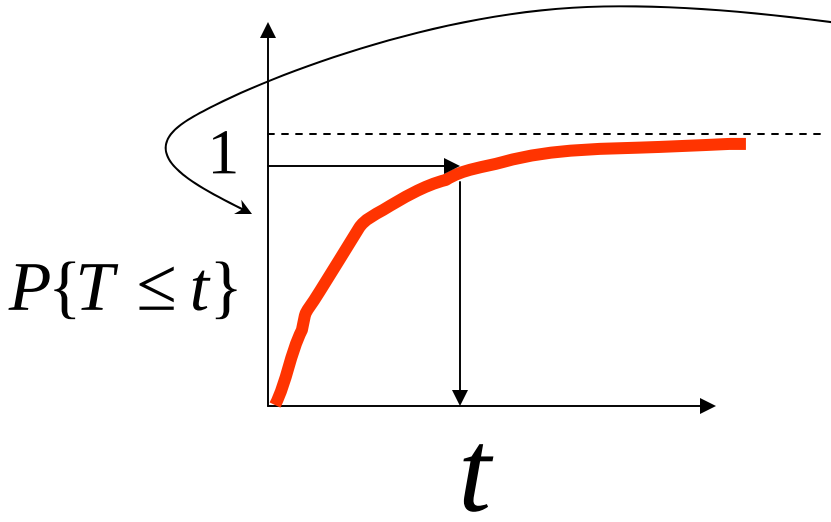
1. Continuous space & Continuous time
2. At every point: right- and left-moving cells
3. Follow a single cell & a population of cells

Velocity Jump Process

$$P\{T \le t\} = 1 - \exp(-\lambda t)$$

$$v = \pm V$$

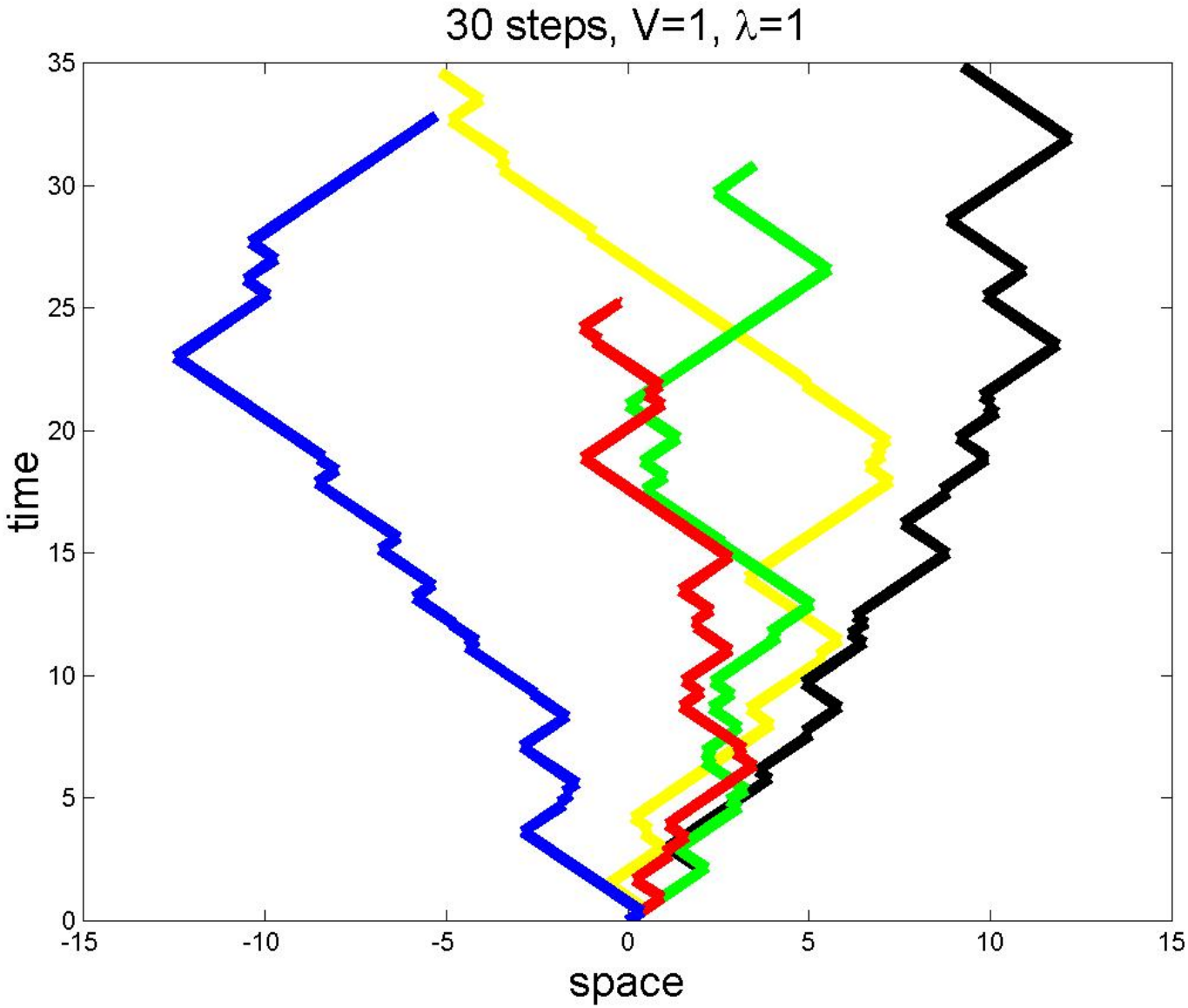
Inversion method



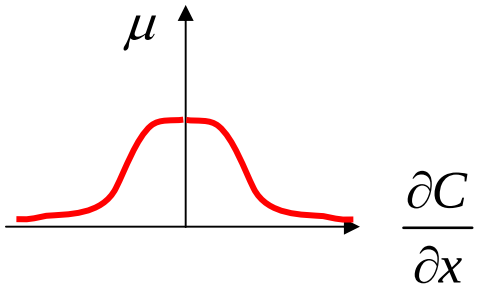
Simulation

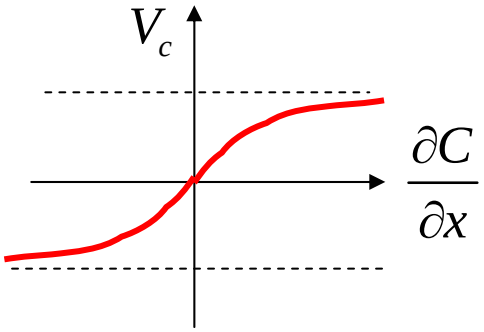
```
λ =1; t=[0];
x=[0]; V=1; STEPS=30
for j=1:5
   for i=1:STEPS;
        T=-log(1-rand(1))/λ;
        N=length(t);
        t=[t;t(N)+T];
        x=[x;x(N)+V*T];
        V=-V;
    end; plot(x,t); hold on;
end;
```

Velocity Jump Process



Flux in a 1D Gradient (4): Analysis





1. Random motility coefficient is a decreasing function of spatial gradient: at large gradients all cells swim in one direction

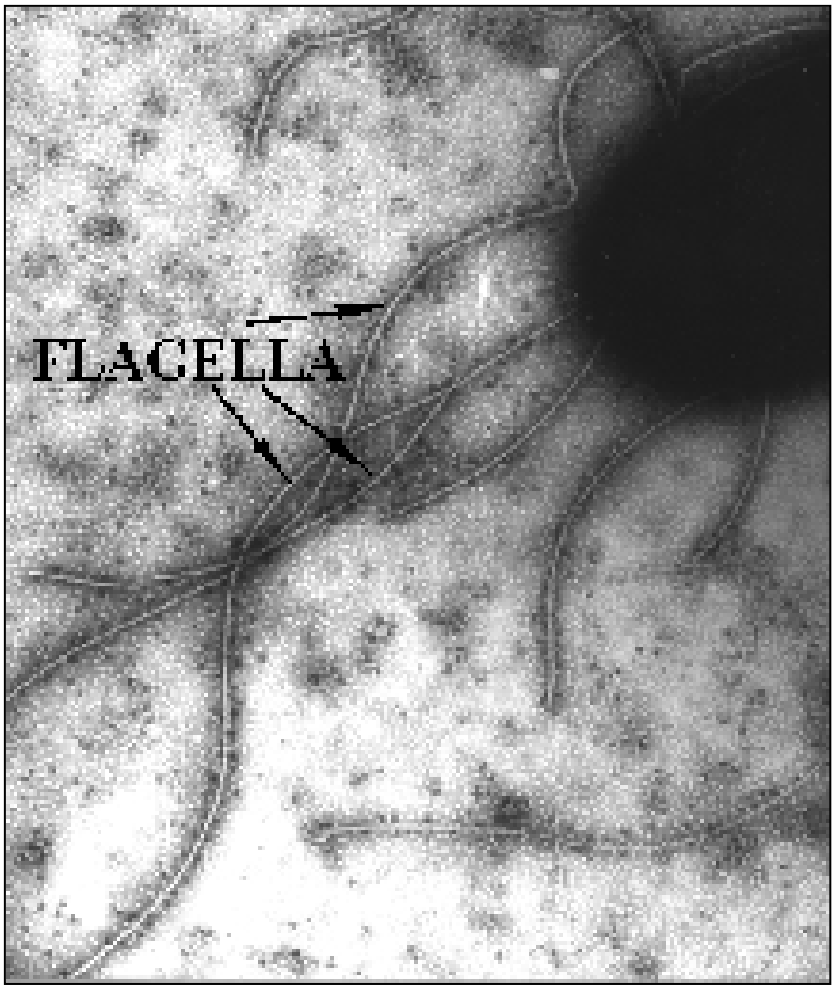
2. Chemotactic velocity has a limiting value: the population can not move faster than the maximal cell speed

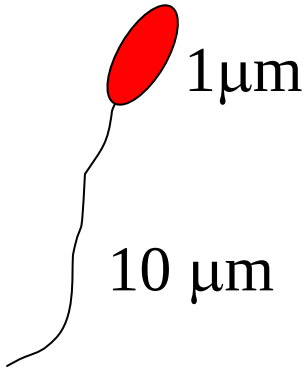
E. Coli swims by rotating its flagella

Flagellar rotation as a means of bacterial motility

1974

Speed: 20-30 μm/s



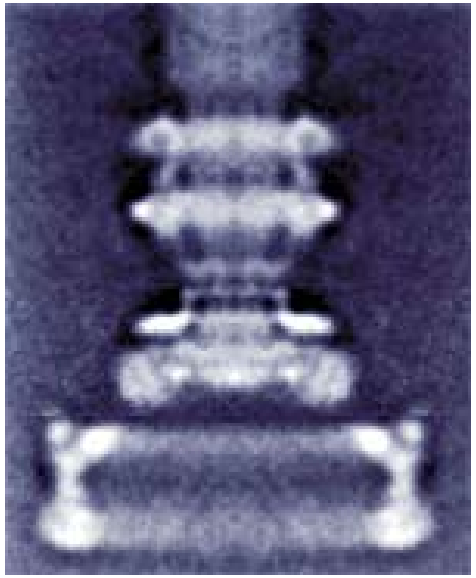


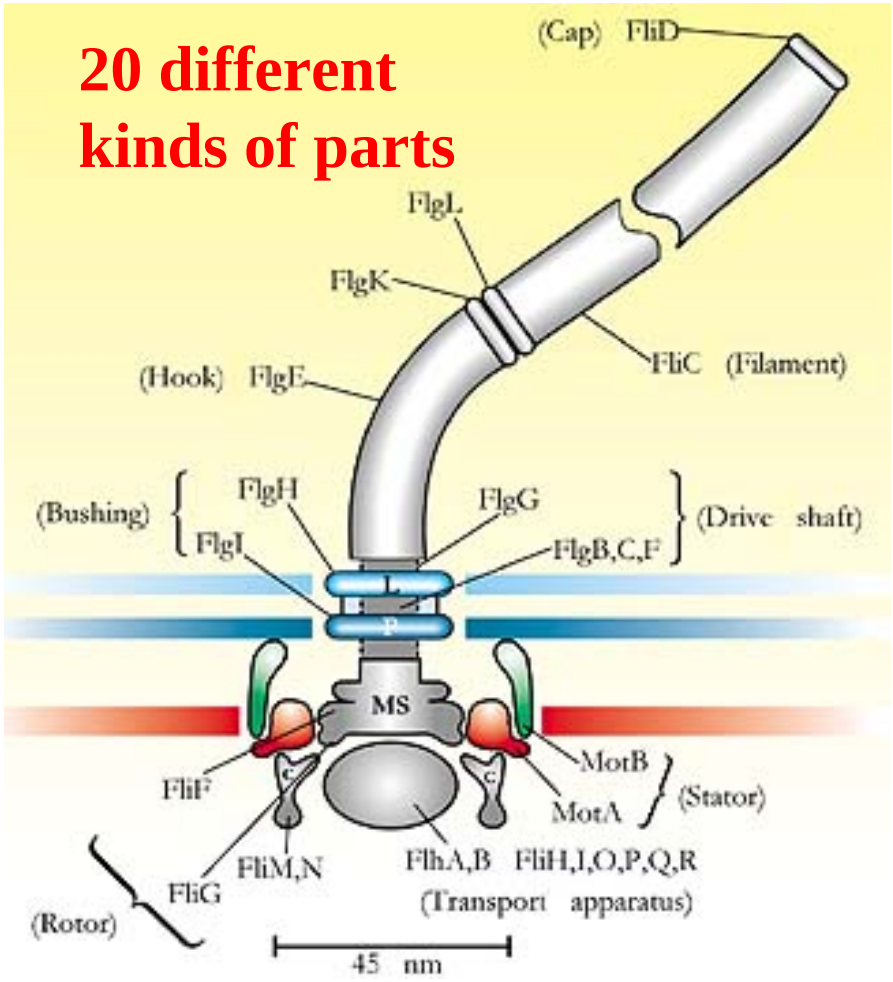
Propulsive unit: a bundle of bacteria

Berg HC Motile behavior of bacteria PHYS TODAY 53 (1): 24-29 JAN 2000

The motor

1974: These observations suggest that the hook is driven in a rotary fashion, probably by the mechanism anchored to cell body at the base of the flagellum.
… the cell has the capacity to vary the direction of the rotation and the speed as well as the frequency of stopping.
(Silverman and Simon, 1994, Nature, 249, 73)





The flagellum is an organelle that has three parts (as figure 2 shows).

There is a basal body consisting of a reversible rotary motor embedded in the cell wall, beginning within the cytoplasm and ending at the outer membrane.

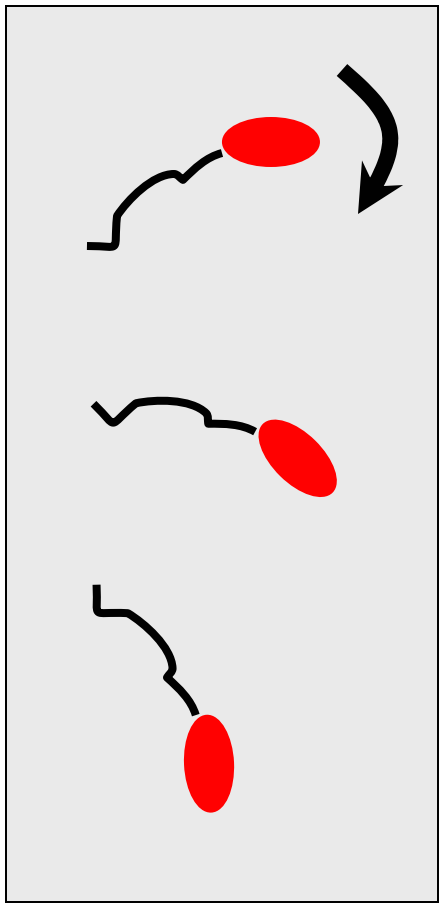
There is a short proximal hook, which is a flexible coupling or universal joint.

And there is a long helical filament, which is a propeller.

Proteins forming the motor have been identified

The motor can rotate in 2 directions – CW and CCW – viewed from the end of the flagella

Experiments with tethered cells: bacterium is attached to a slide. The whole cell rotates. (Silverman and Simon, Adler et al, 1974, "Nature")



<u>Change in direction of flagellar rotation is the basis of the chemotactic response in E. Coli, 1974, Adler et al</u>

• 1972: Chemotaxis is produced by variation in the tumbling frequency

• 1974: Tumbling frequency is produced by the CW rotation of the flagella

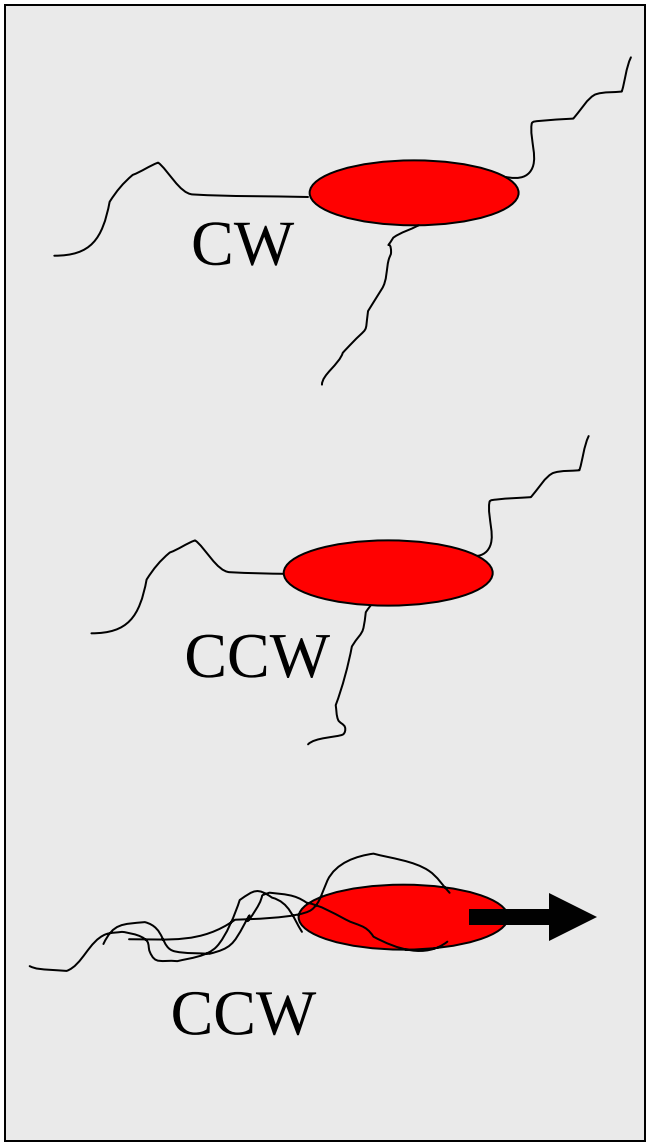
• Rotation bias is affected by chemicals
• Tumbling frequency is affected by chemicals

CW rotation – tumble
CCW – swimming

Same pattern of attractant responses for CW/CW and tumble/run

But this is for one flagella.
The propulsive unit is a bundle
How does it form?

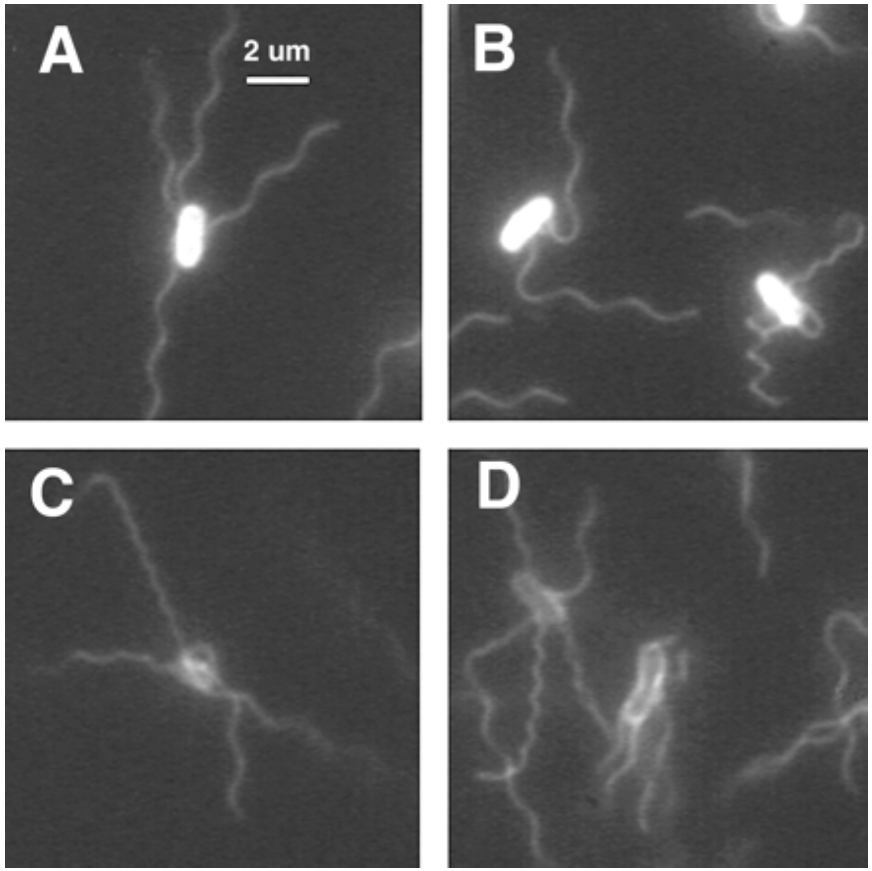
Hydrodynamic forces can bundle individual flagella
(large reduction in power dissipation for synchronous rotation within a bundle)
Many other examples of hydrodynamics-based synchronization



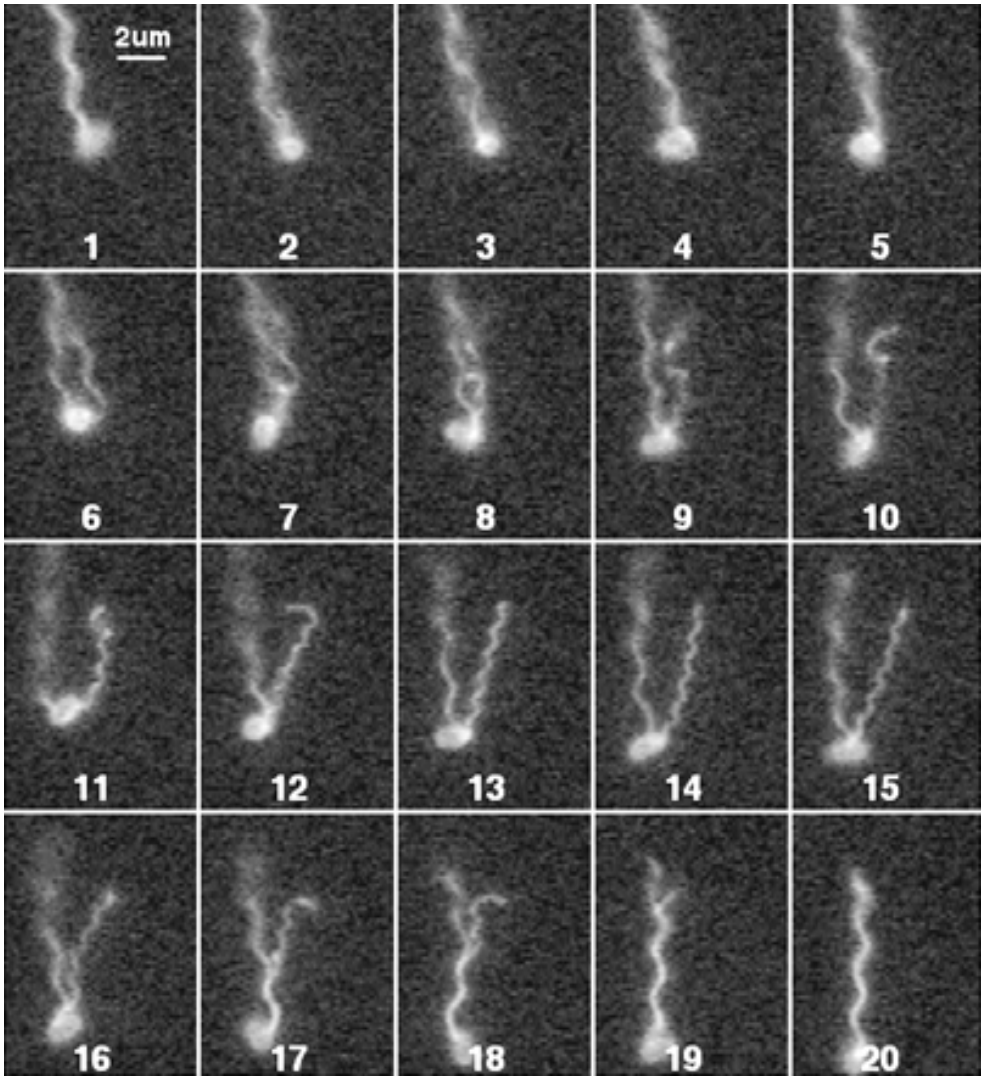
Is it just a theory ?

Real-time imaging of fluorescent flagellar filaments

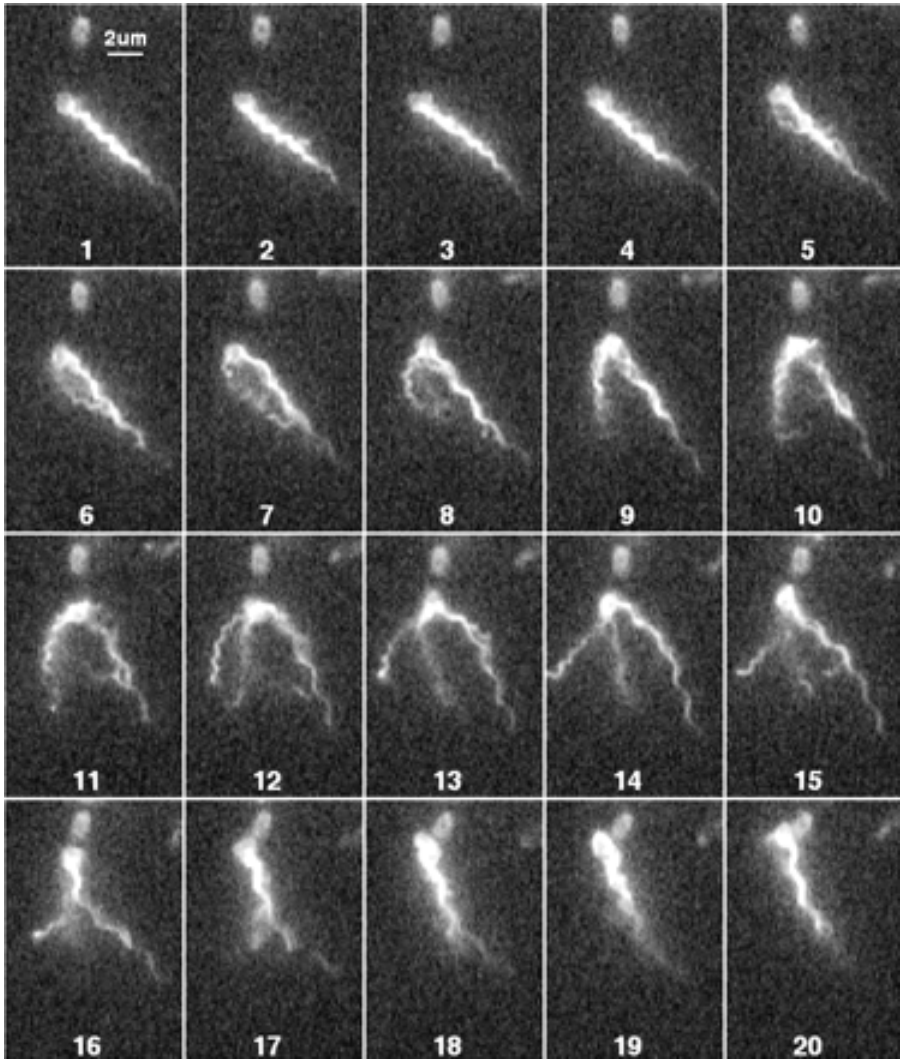
Turner, Ryu, and Berg, J. Bacteriology, 182, 10, 2783, 2000



2 flagella

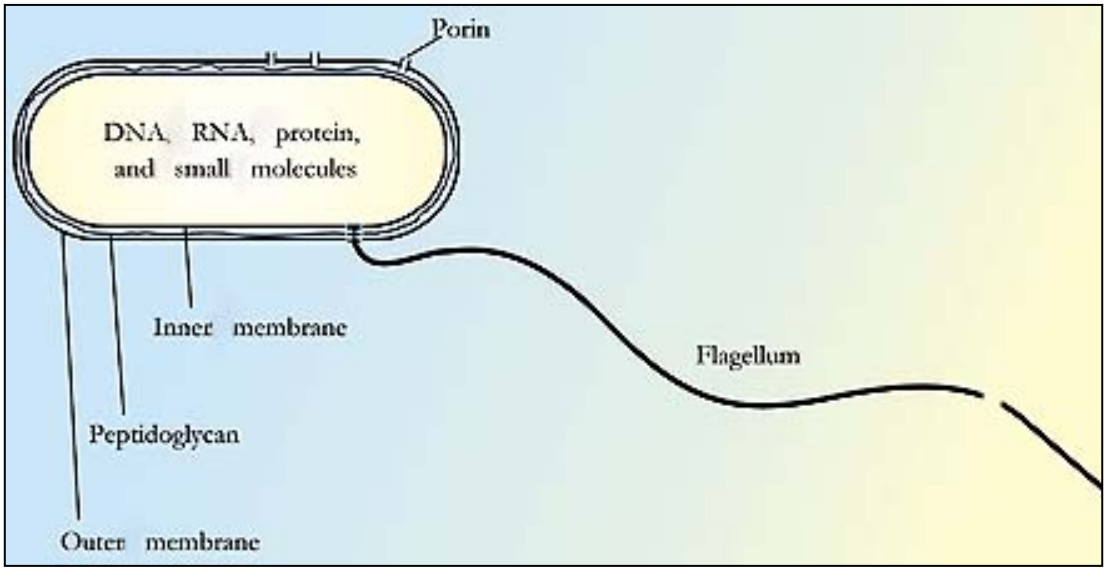


Several flagella



- **Not all flagella have to rotate CCW for the cell to “run”**
- **Only several flagella can rotate CW to cause tumbling**
- **Different motors behave “independently”**

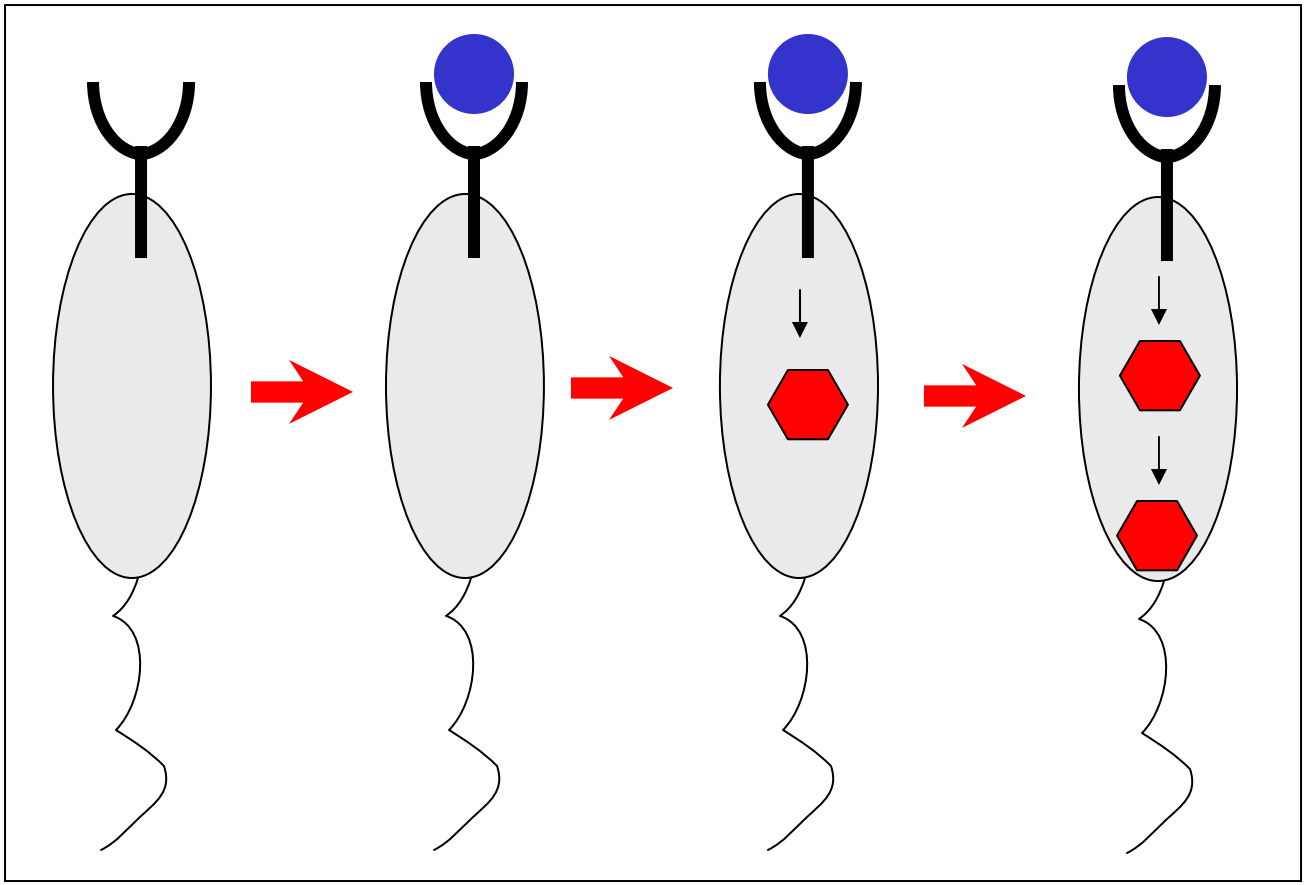
Attractants/repellants modify rotational bias
How is it accomplished?



Look inside the cell

What happens when ligand binds?

- Receptor directly linked to the motor – NO
- Electric signal generated – NO
- Diffusing messenger (~1985)



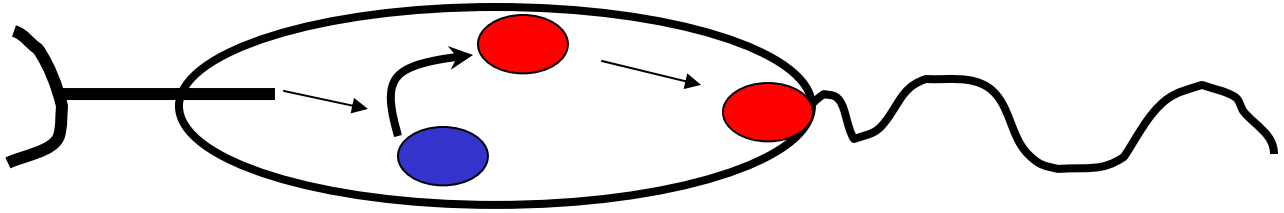
Signal transduction

Concentration of an active form of a cytoplasmic protein regulates the rotational bias of the motor

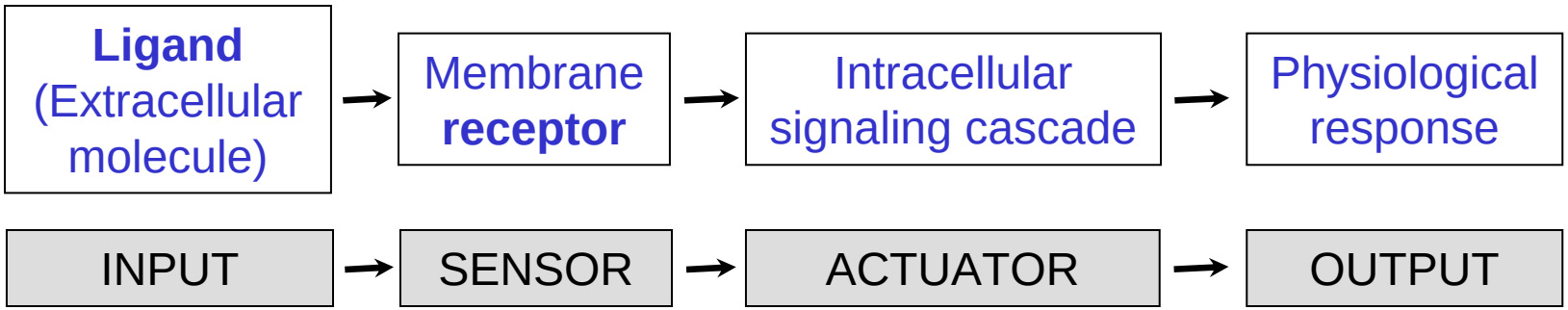
CheY protein

CheY protein phosphorylated

Small molecule: 14 -kDa

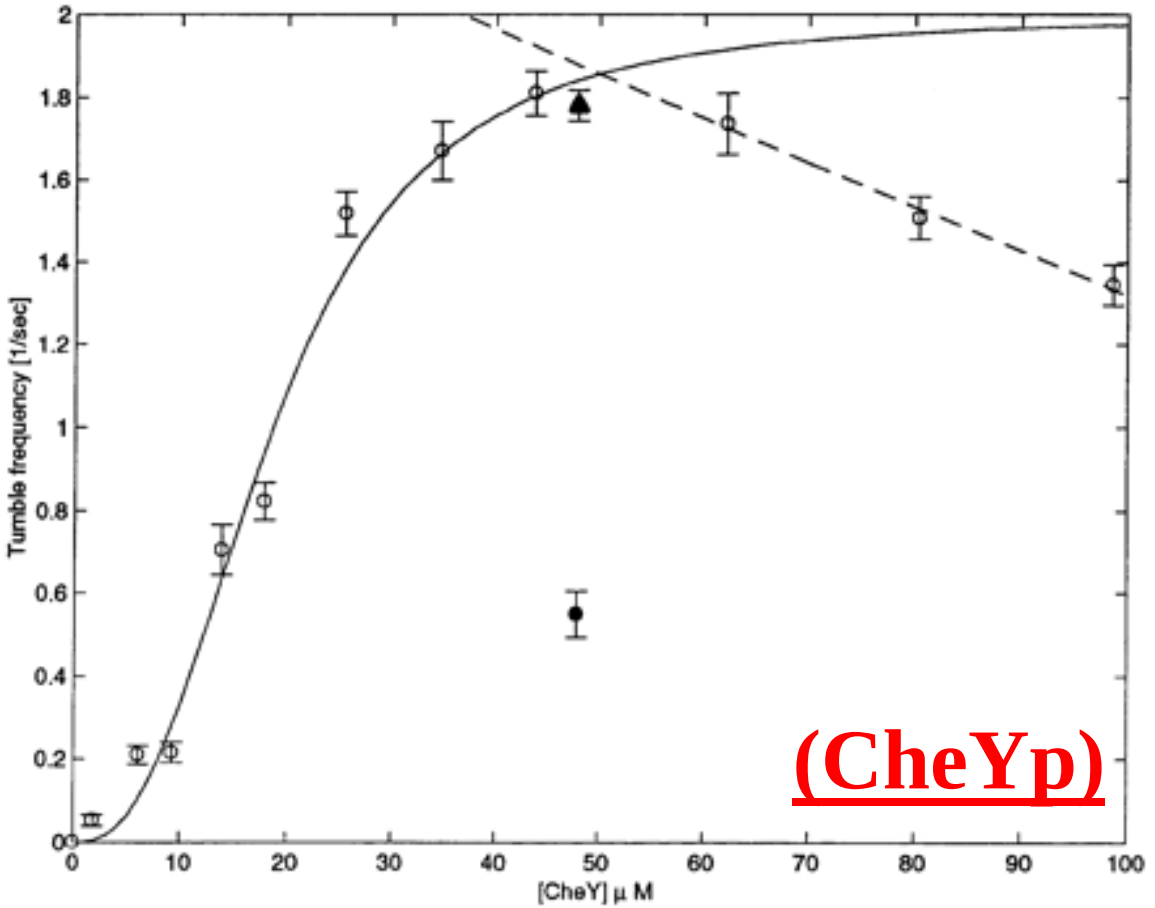


Time to traverse the cytoplasm ?



Input/output behavior quantified:
Motor bias = F( )

Tumbling frequency



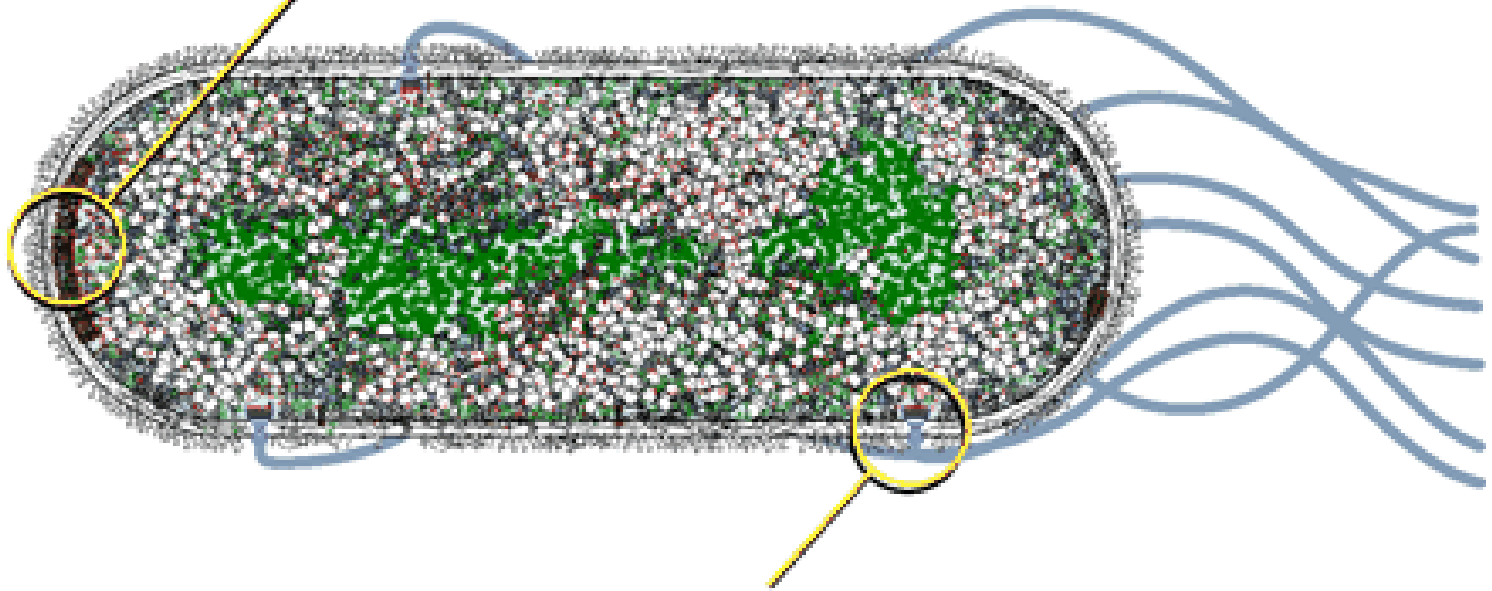
(CheYp)

Alon U, Camarena L, Surette MG, Aguera y Arcas B, Liu Y, Leibler S, Stock JB.
Response regulator output in bacterial chemotaxis.
EMBO J. 1998 Aug 3;17(15):4238-48.

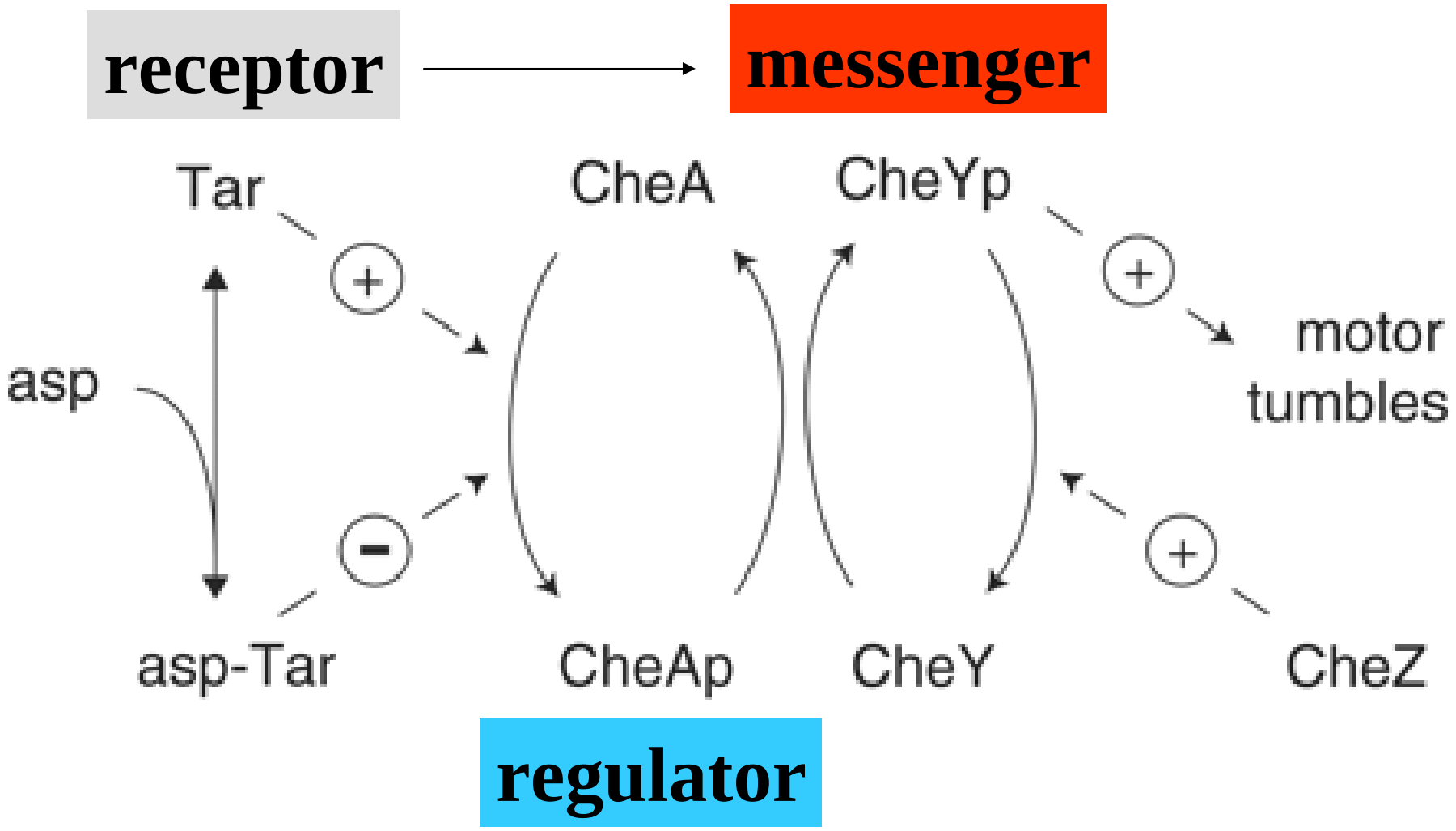
Scharf BE, Fahrner KA, Turner L, Berg HC.
Control of direction of flagellar rotation in bacterial chemotaxis
Proc Natl Acad Sci U S A. 1998 Jan 6;95(1):201-6.

Gradient of chemical -> Flux of bacteria
CheY concentration -> statistics of motor reversals

Swimming bacterium encounters aspartate.
Occupancy of the receptor Tar increases.

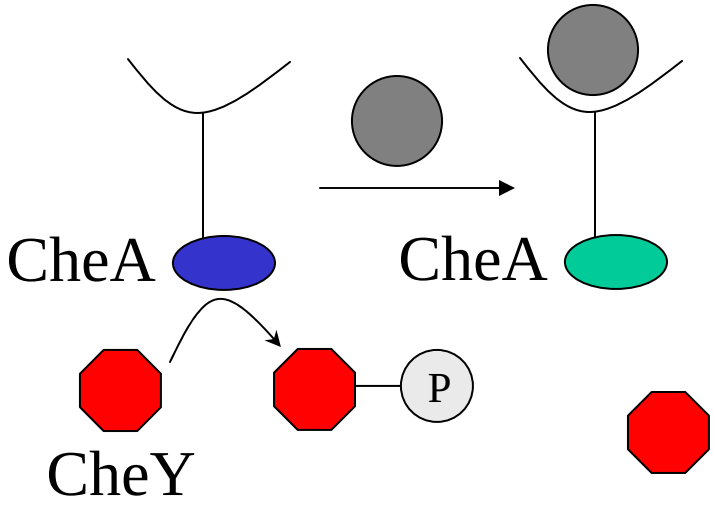


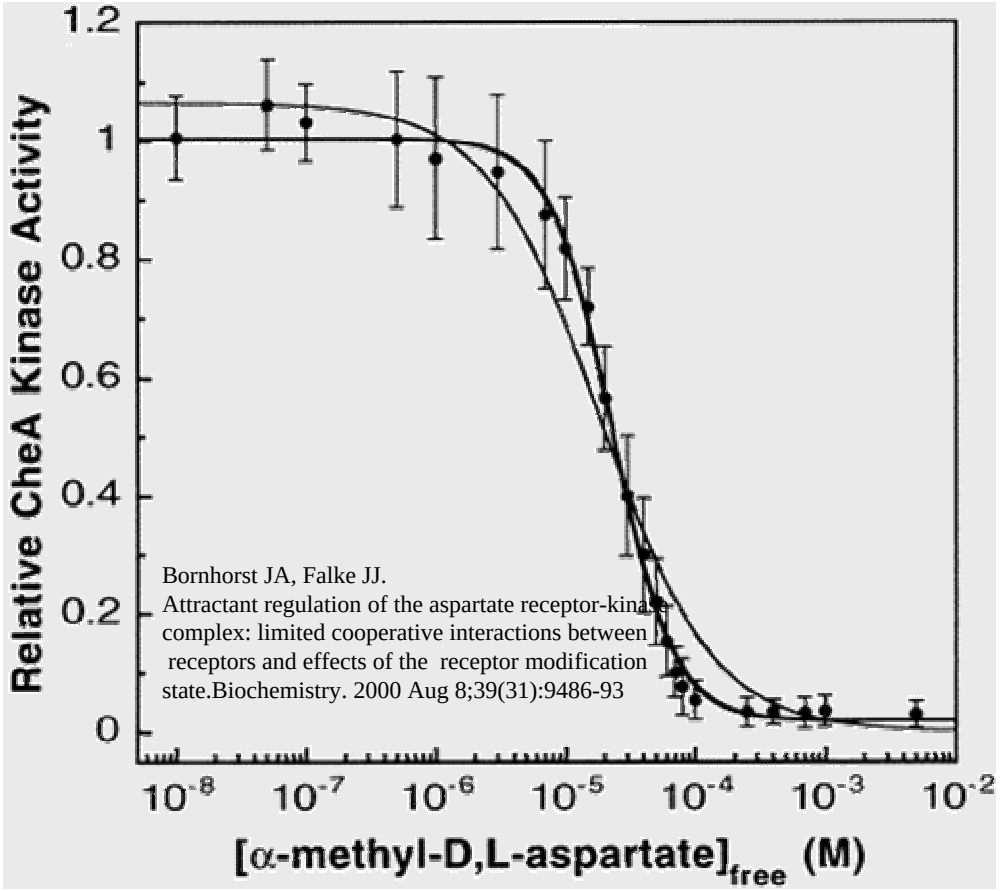
Concentration of cytosolic CheYp falls.
Probability of motor CCW rotation (bias) increases.


receptor
messenger
Tar
CheA
CheYp
asp
motor
tumbles
asp-Tar
CheAp
CheY
CheZ
regulator

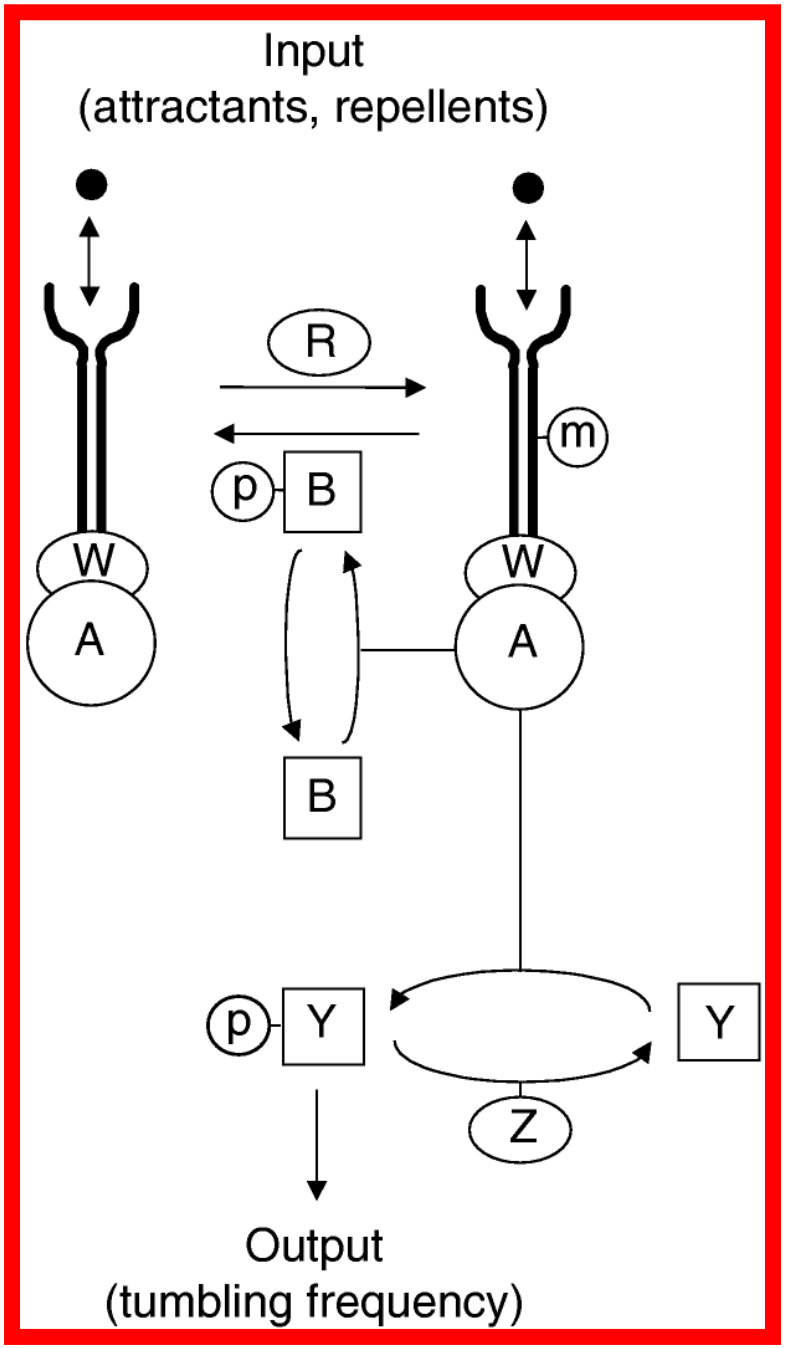
CheY is phosphorylated by a receptor-linked kinase (**regulator**)

Ligand binding negatively regulates kinase activity

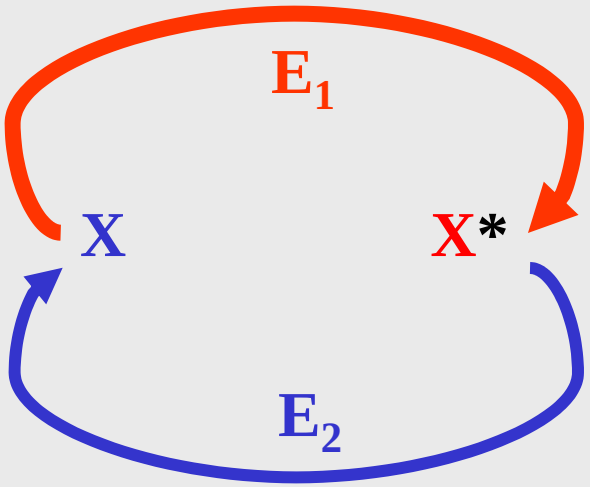


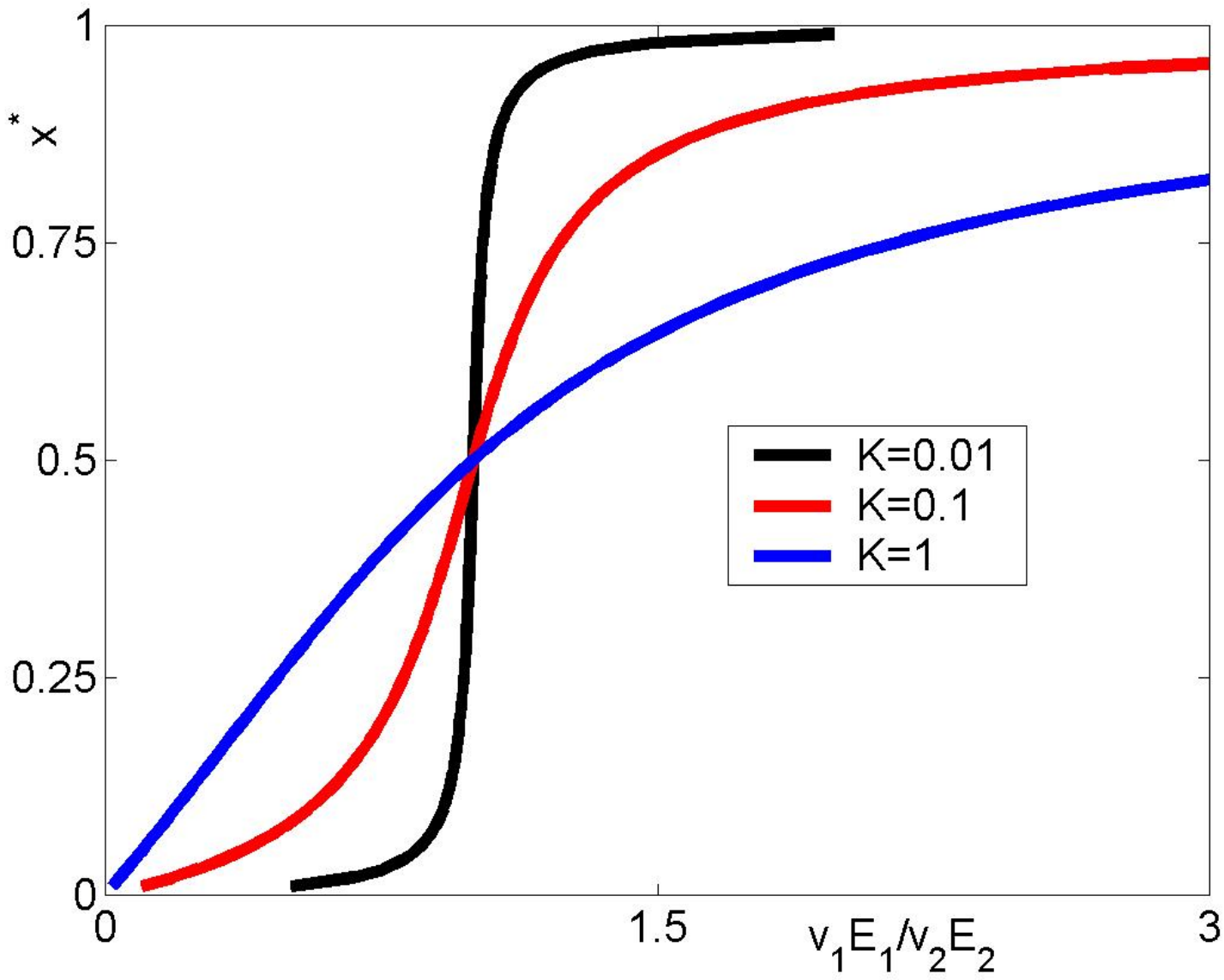


This is <u>only an input</u> to the network that regulates CheY-P



A common motif:




1
0.75
0.5
0.25
0
0
1.5
3
x*
v1E1/v2E2
K=0.01
K=0.1
K=1

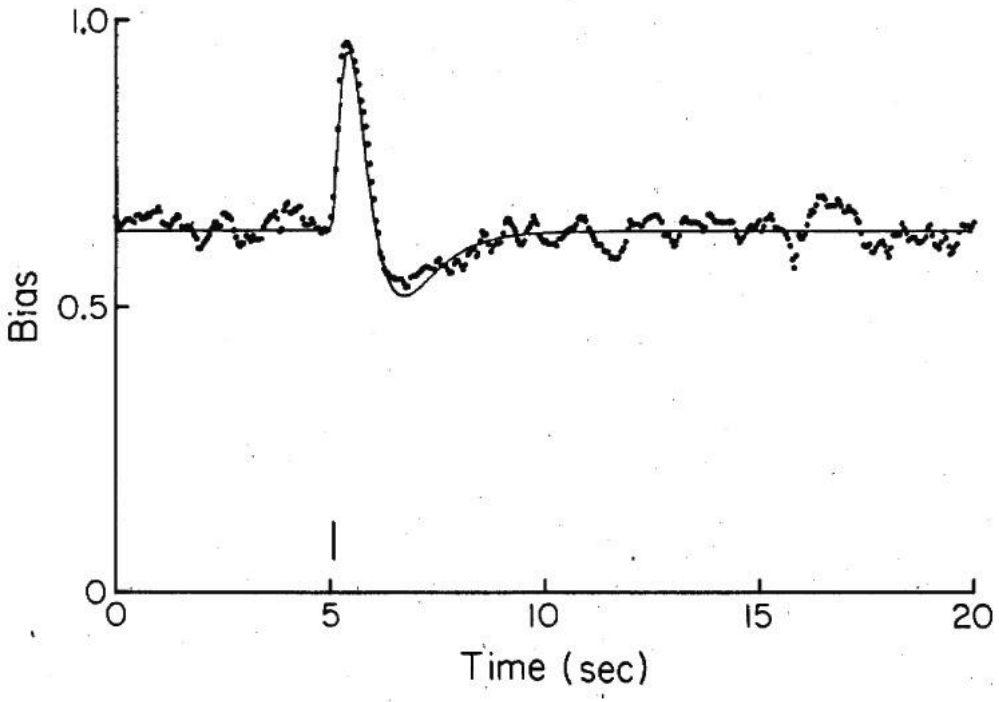


FIG. 1. Impulse response to attractant in wild-type cells. The dotted curve is the probability, determined from repetitive stimulation, that tethered cells of strain AW405 spin CCW when exposed to pulses of L-aspartate or α-methyl-DL-aspartate beginning at 5.06 sec (vertical bar). The smooth curve is a fit to a sum of exponentials (see text). For methods, see refs. 14 and 16. Pipettes containing aspartate (1 mM) were pulsed for 0.02 sec at -25 to -100 nA, and pipettes containing methylaspartate (1–3 mM, with 1.6 mM in the bath) were pulsed for 0.12 sec at -100 nA, both at 32°C. Some pipettes containing 1–7 mM methylaspartate were pulsed for 0.03–0.12 sec at -50 to -100 nA at 22°C. The curve was constructed from 378 records comprising 7566 reversals of 17 cells. Points were determined every 0.05 sec.

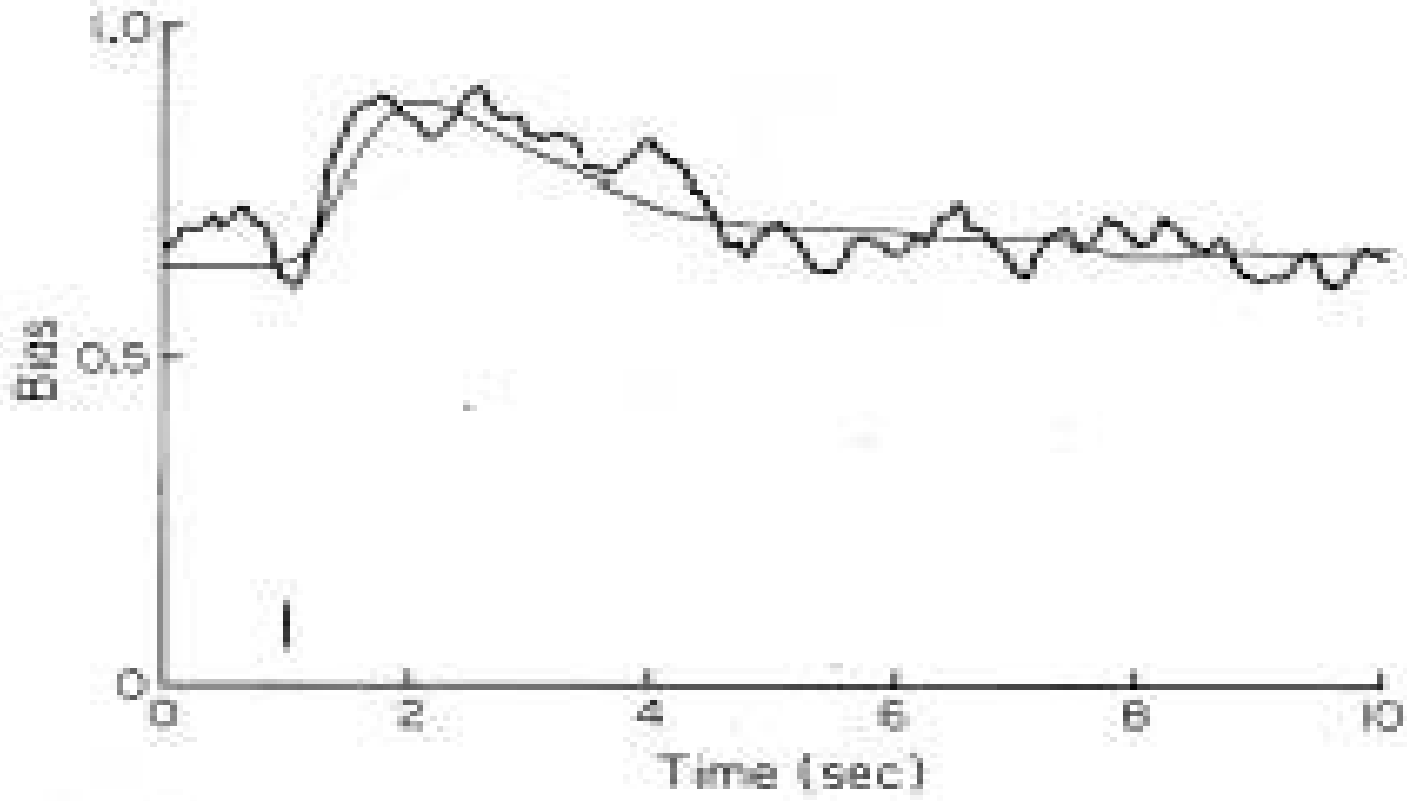
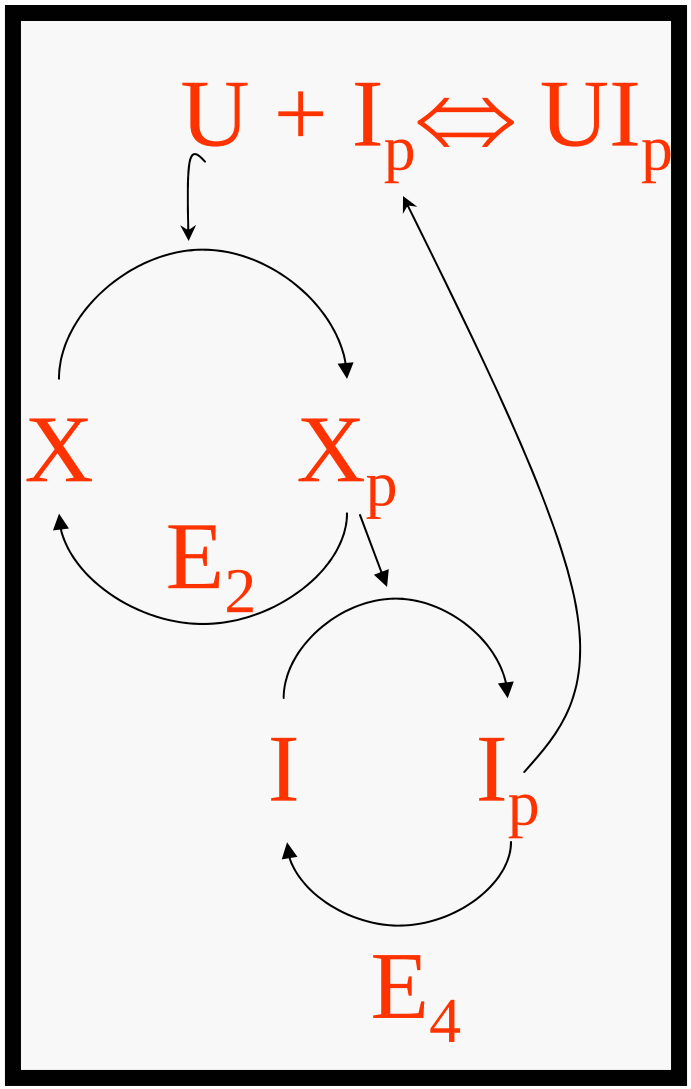


FIG. 2. Step response to attractant in wild-type cells. The thick rve is the probability that cells of strain AW405 spin CCW when



$$\frac{dX_p}{dt} = \frac{u}{1+G*I_P}\frac{X}{K_1+X} - \frac{V_2 X_p}{K_2+X_p}$$

$$\frac{dI_p}{dt} = \frac{V_3 X_p I}{K_3+I} - \frac{V_4 I_p}{K_4+I_p}$$

$1 = I + I_P$, K_3 & $K_4 << 1$

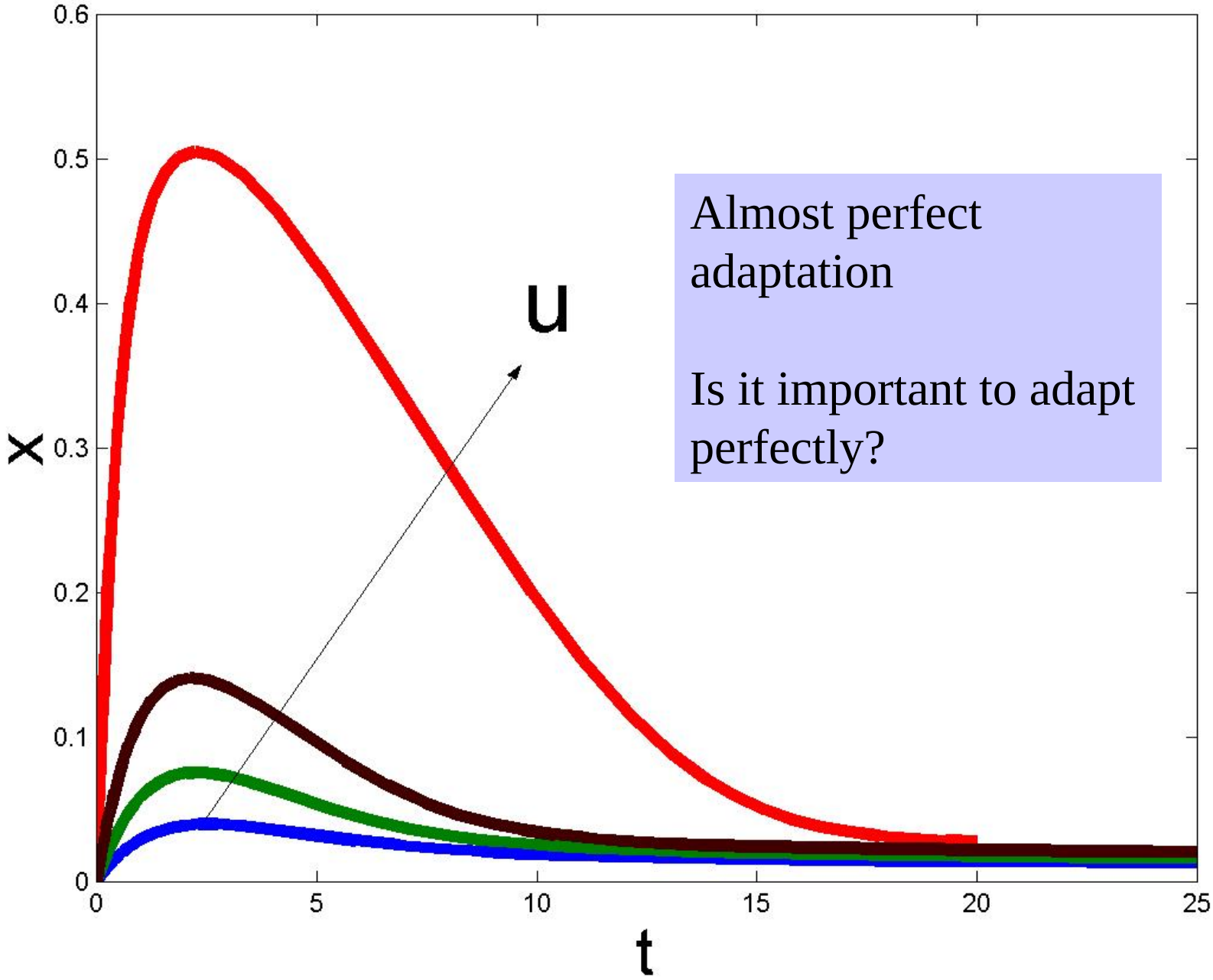
$1 = X + X_P$

At steady state:

$X_P \approx \frac{V_4}{V_3}$ independently of u, K_1, K_2

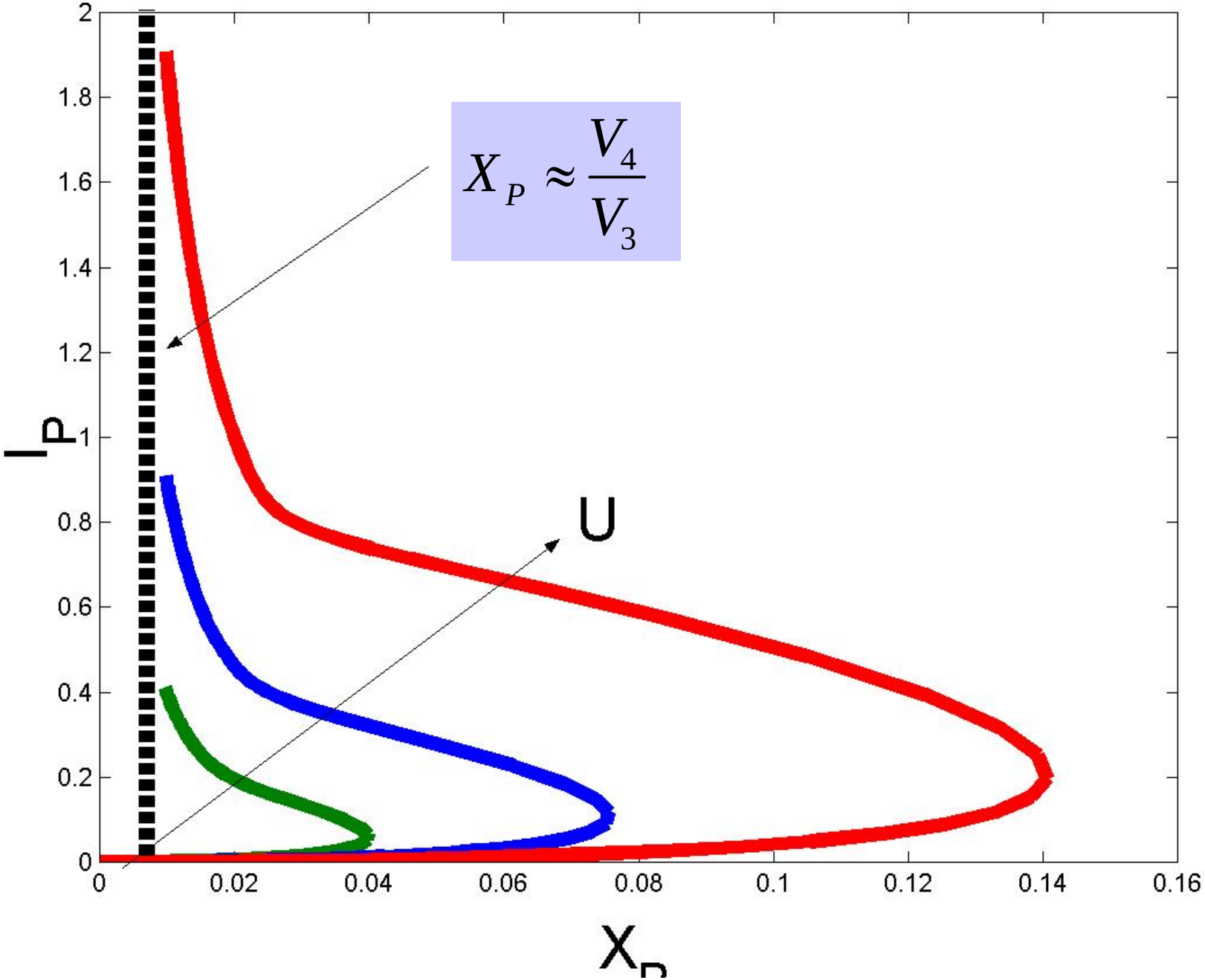
make $\frac{V_4}{V_3}$ small

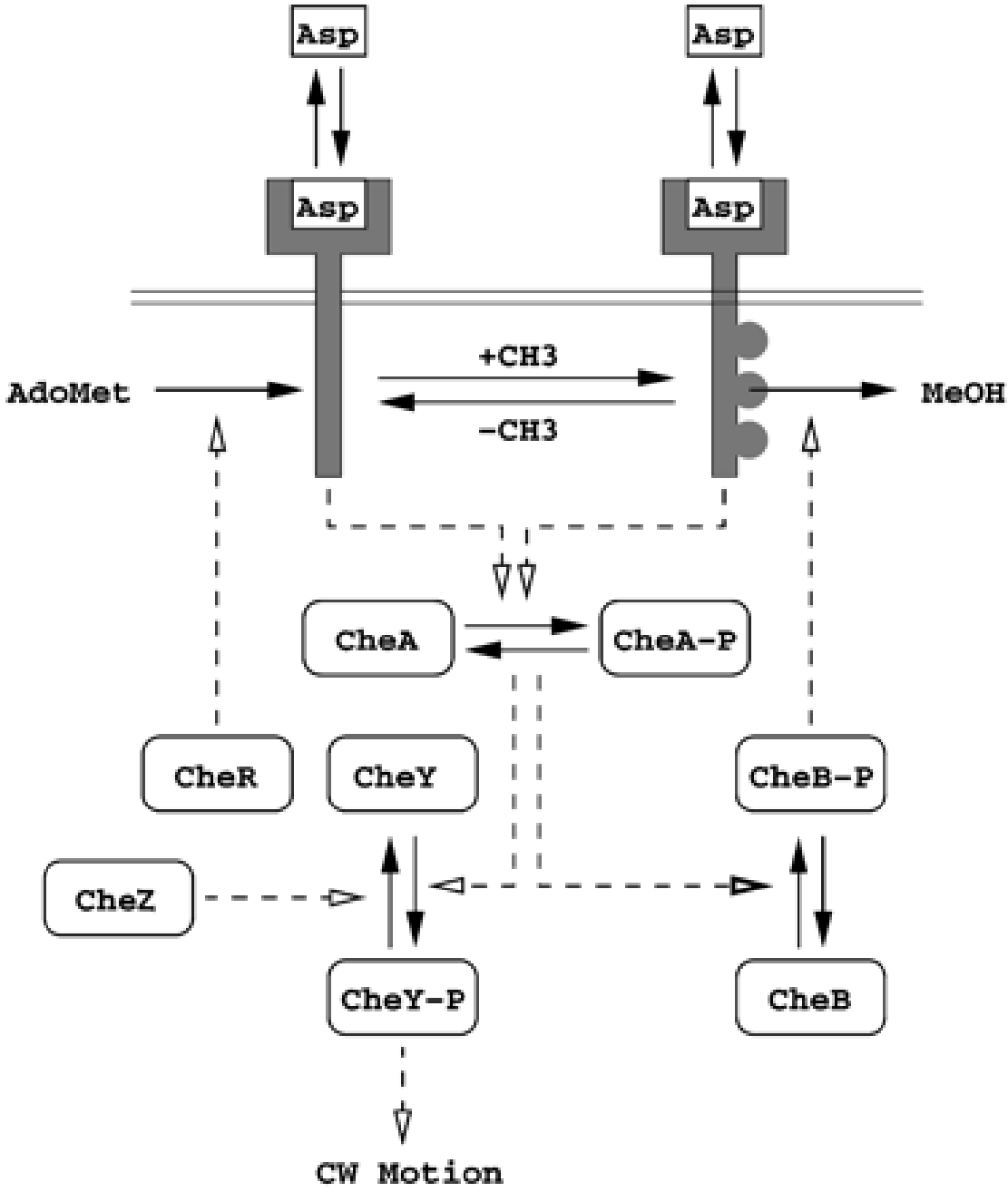
```
function y = feedback(t,x,flag,p)


u=p(1); g=p(2);
K=0.1; I=x(2); X=x(1);

y(1,1) = 0.1*((u/(1+p(2)*I))*(1-X)/(K+1-X) - X/(K+X));
y(2,1) = (X - 0.01*I/(0.01+I));

return;

U=[0,0.5,1,2];

for i=1:length(U)

   x0=[0;0]; u=U(i); g=10.0; P=[u;g];
   [t,y]=ode23s('feedback',[0,7000],x0,[],P);
   plot(y(:,1),y(:,2)); hold on;

end;
```

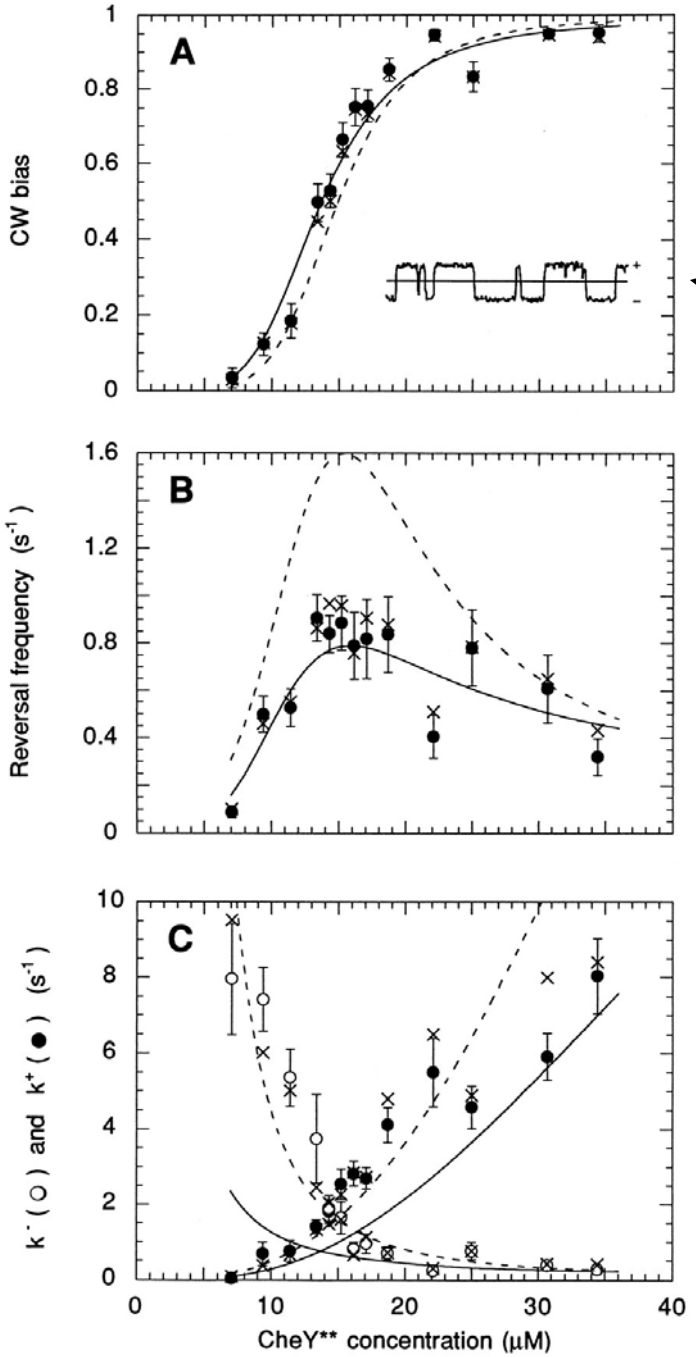


Almost perfect adaptation

Is it important to adapt perfectly?

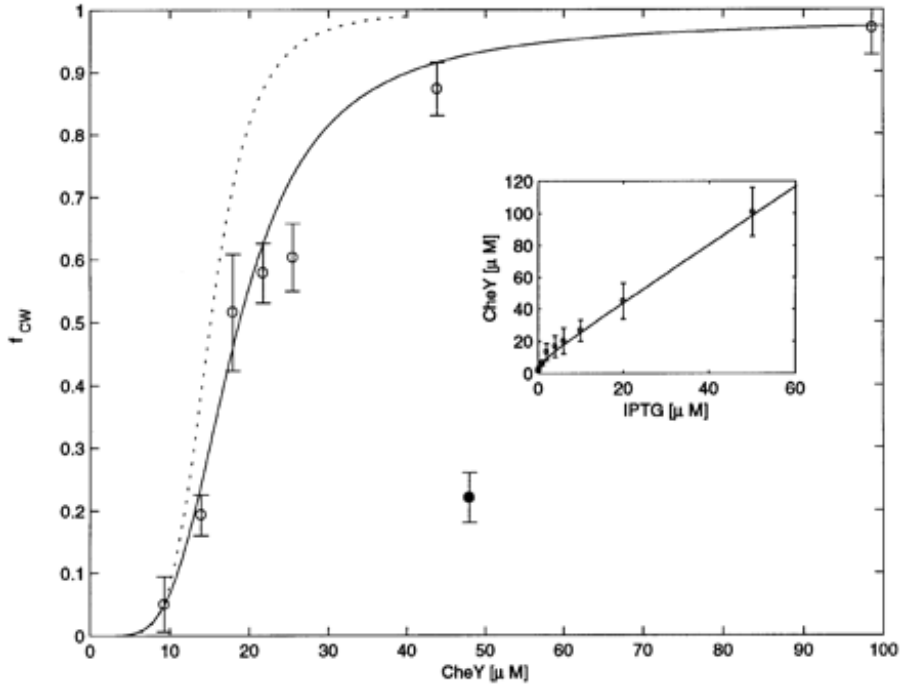

$X_P \approx \frac{V_4}{V_3}$
U
I_P
X_P
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16


Asp
Asp
Asp
Asp
+CH3
-CH3
AdoMet
MeOH
CheA
CheA-P
CheR
CheY
CheB-P
CheZ
CheY-P
CheB
CW Motion



Input/output behavior was quantified:
Motor bias = F(●)

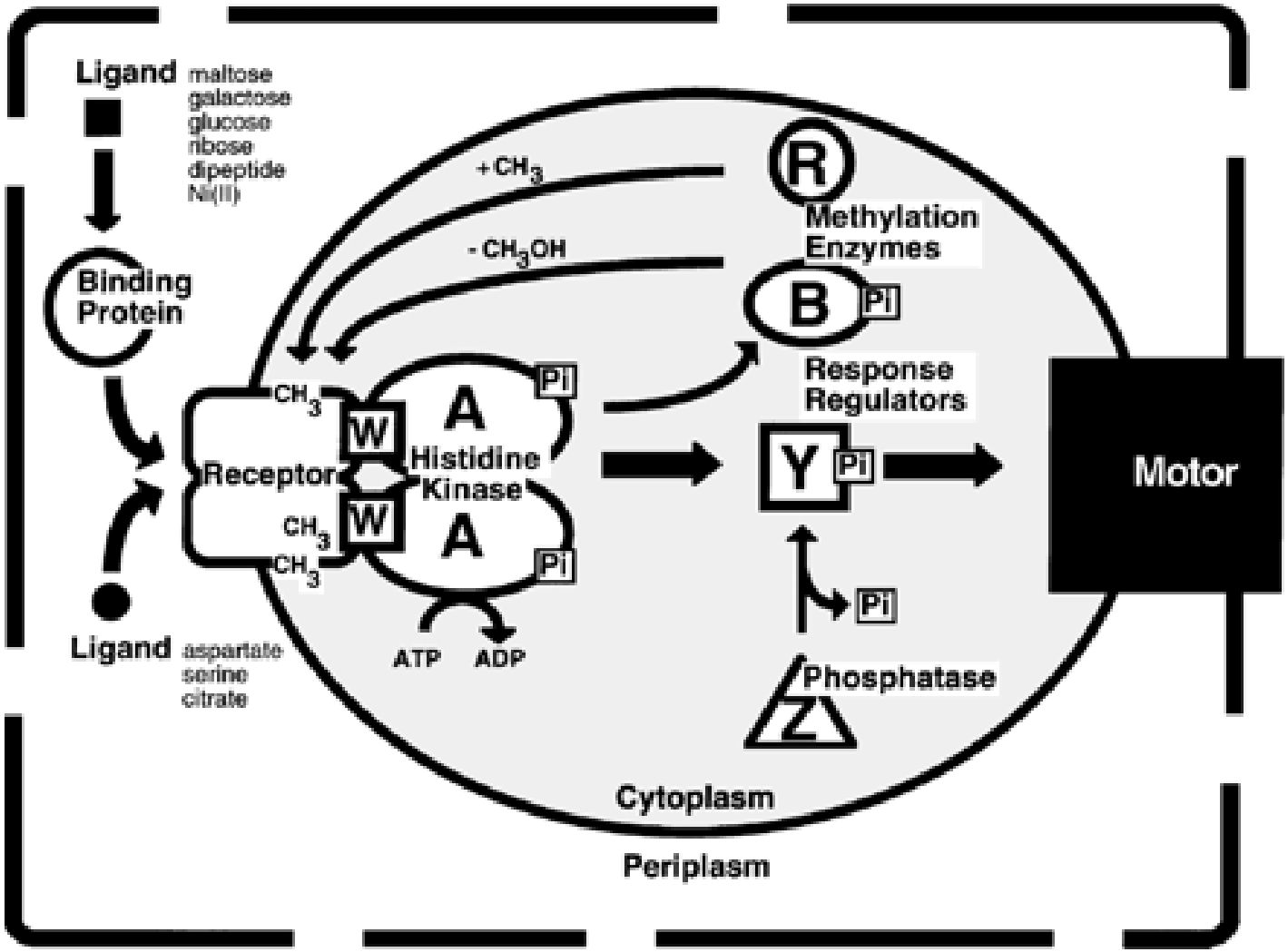
1998

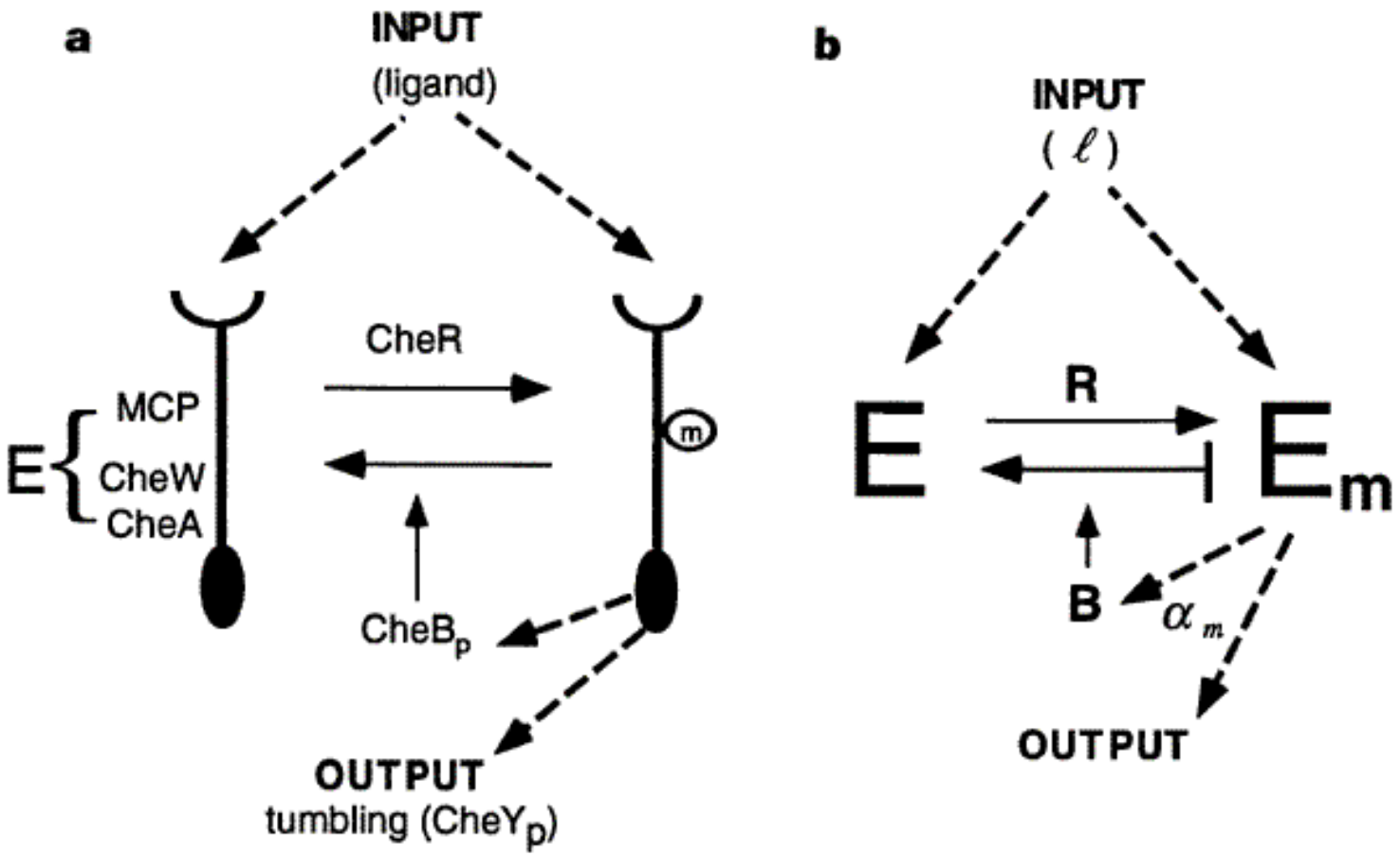


Alon U, Camarena L, Surette MG, Aguera y Arcas B, Liu Y, Leibler S, Stock JB.
Response regulator output in bacterial chemotaxis.
EMBO J. 1998 Aug 3;17(15):4238-48.

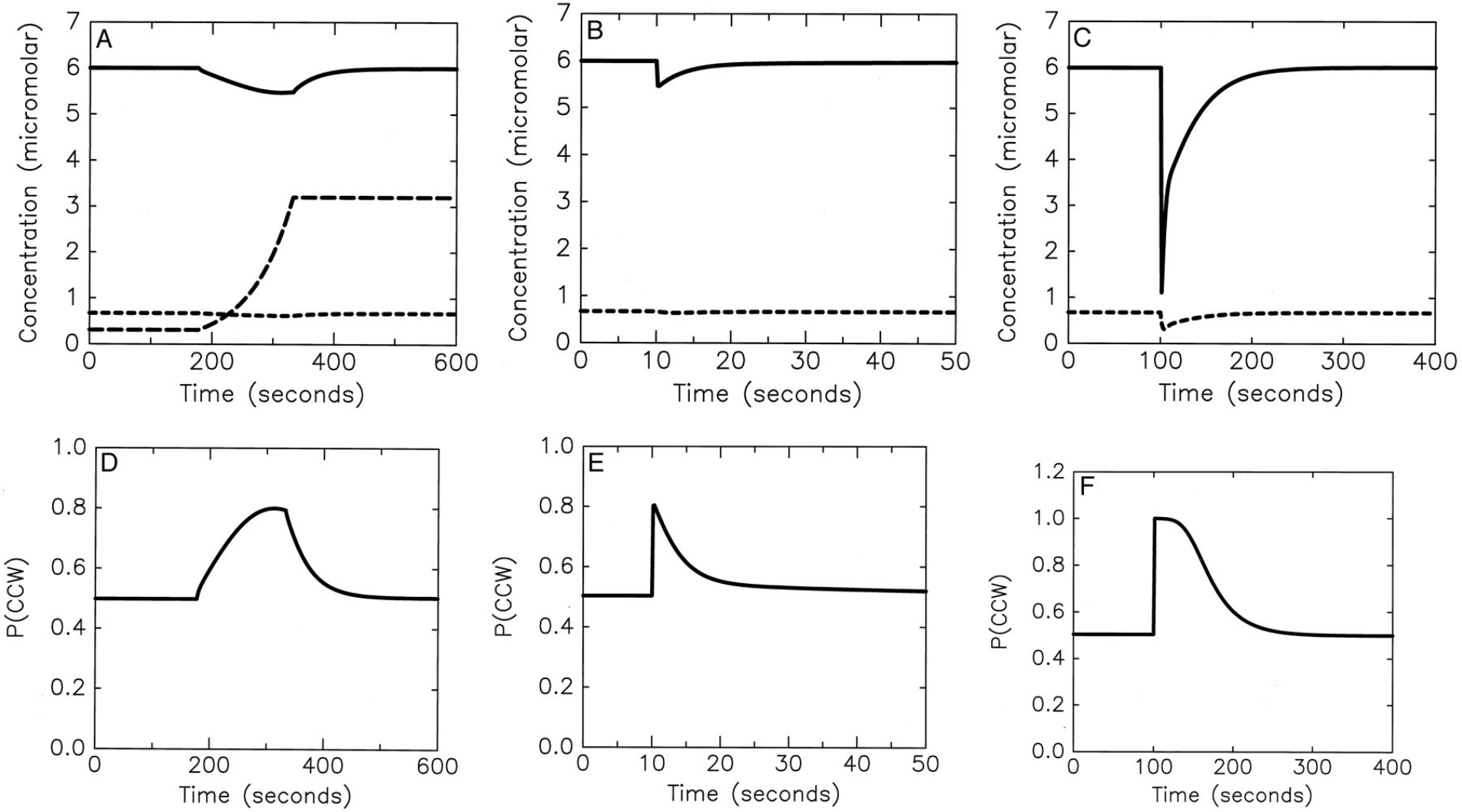
Scharf BE, Fahrner KA, Turner L, Berg HC.
Control of direction of flagellar rotation in bacterial chemotaxis
Proc Natl Acad Sci U S A. 1998 Jan 6;95(1):201-6.

Chemotaxis Network

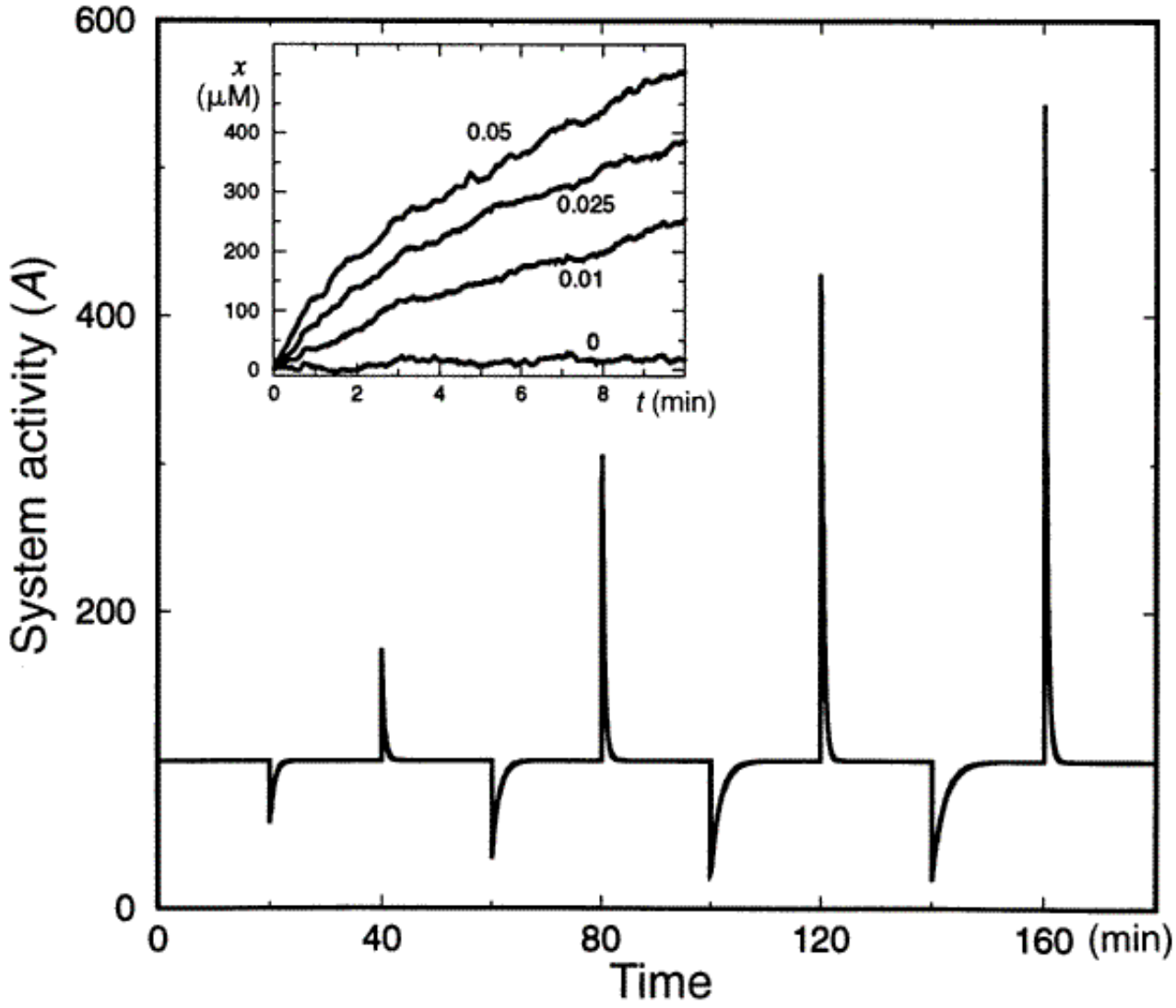



a
INPUT
(ligand)
CheR
MCP
E
CheW
CheA
m
CheB$_p$
OUTPUT
tumbling (CheY$_p$)
b
INPUT
(ℓ)
R
E
E$_m$
B
α_m
OUTPUT

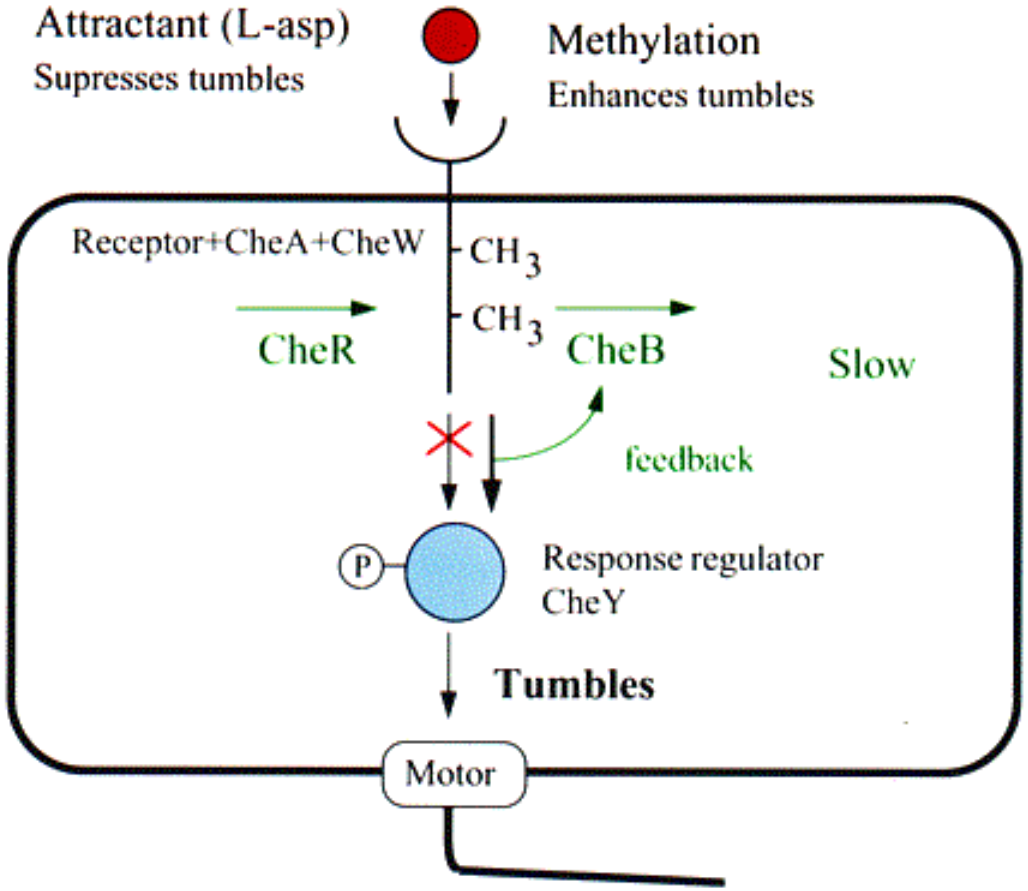
Spiro, Othmer, Parkinson, 1997



Bacterial chemotaxis became a honey pot for modelers

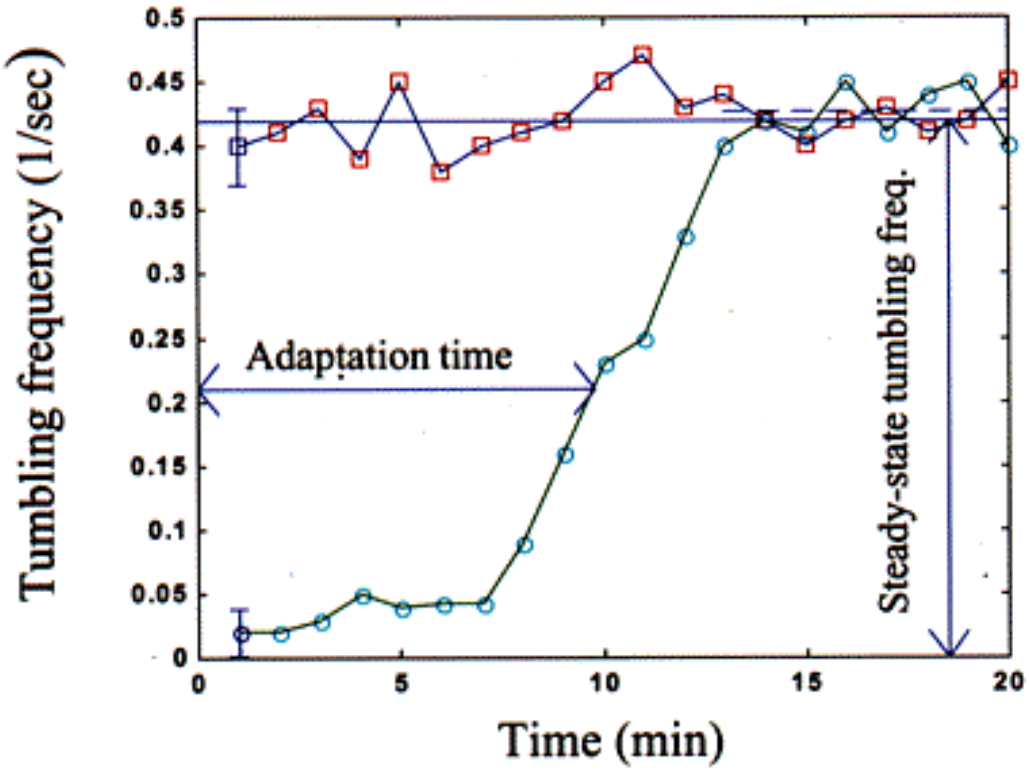


Adaptation is Precise



http://online.itp.ucsb.edu/online/infobio01/alon1/oh/116.html

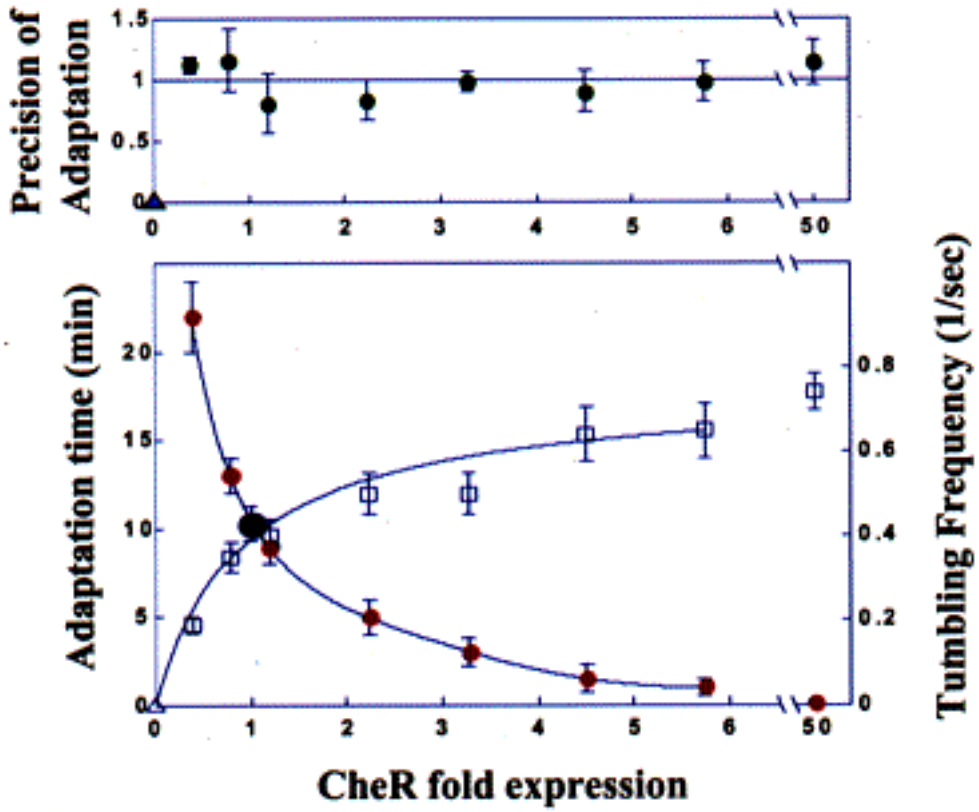
Response and adaptation to attractant



E. coli RP437

http://online.itp.ucsb.edu/online/infobio01/alon1/oh/116.html

Effect of varying CheR concentration

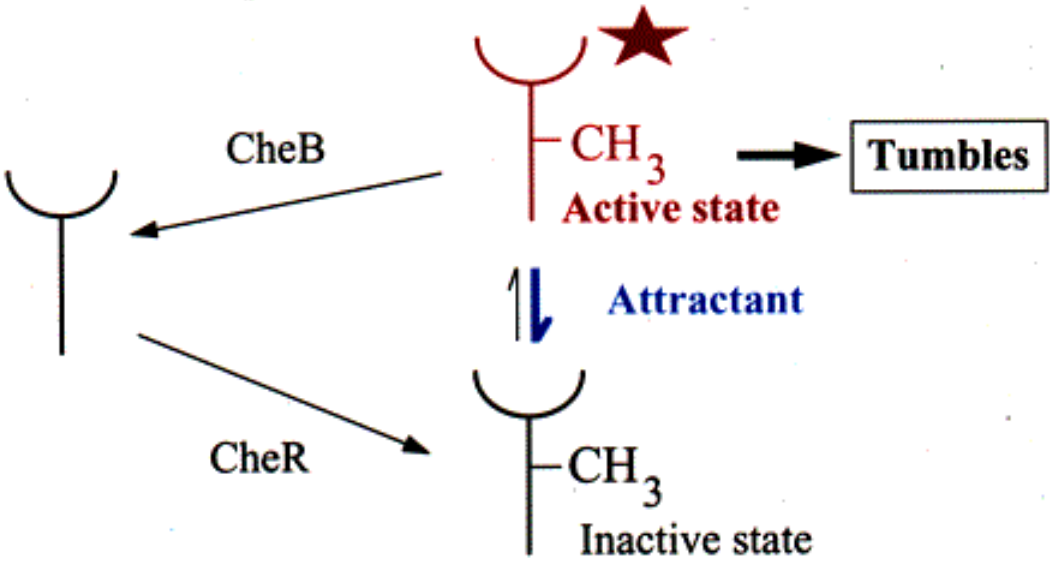


RP4968(ΔcheR) + p lac-CheR

http://online.itp.ucsb.edu/online/infobio01/alon1/oh/116.html

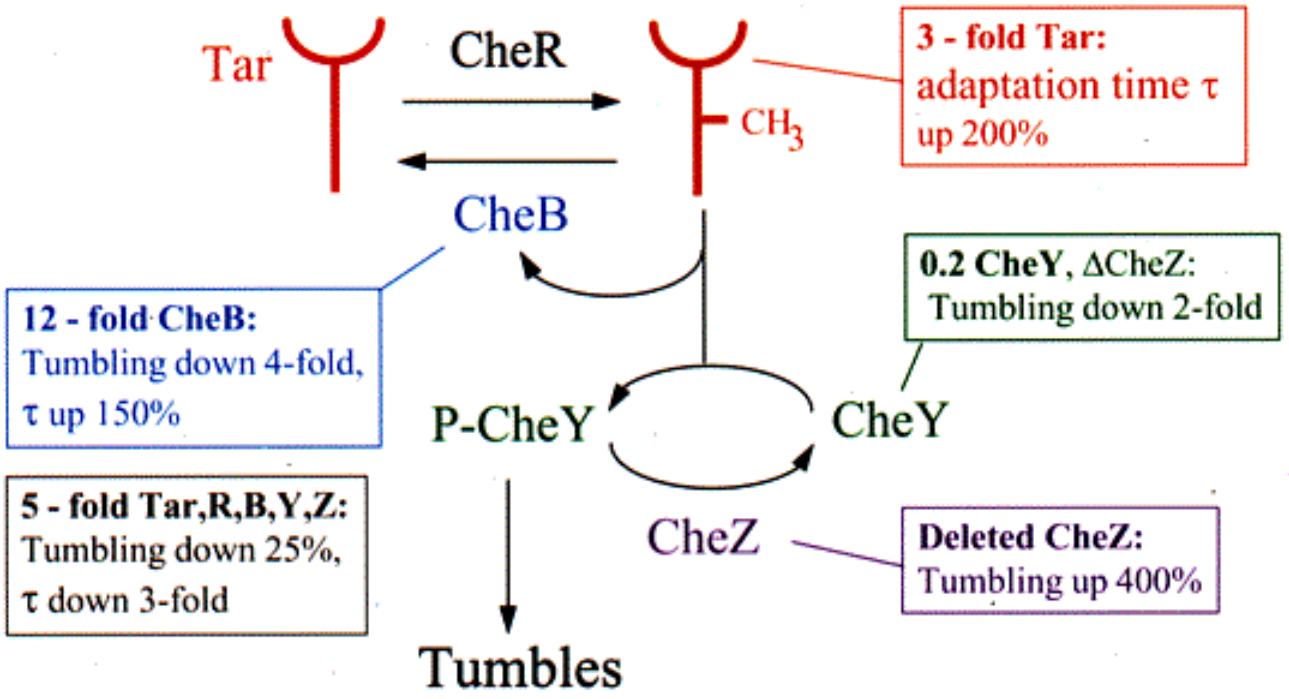
Robust Mechanism for Adaptation

Barkai & Leibler 1997



Two-state receptors: Asakura, Honda 1984.
Stock, Surette 1996.

Varying different proteins in the network



Adaptation precise to within 10% in all cases

http://online.itp.ucsb.edu/online/infobio01/alon1/oh/116.html

The gain is huge

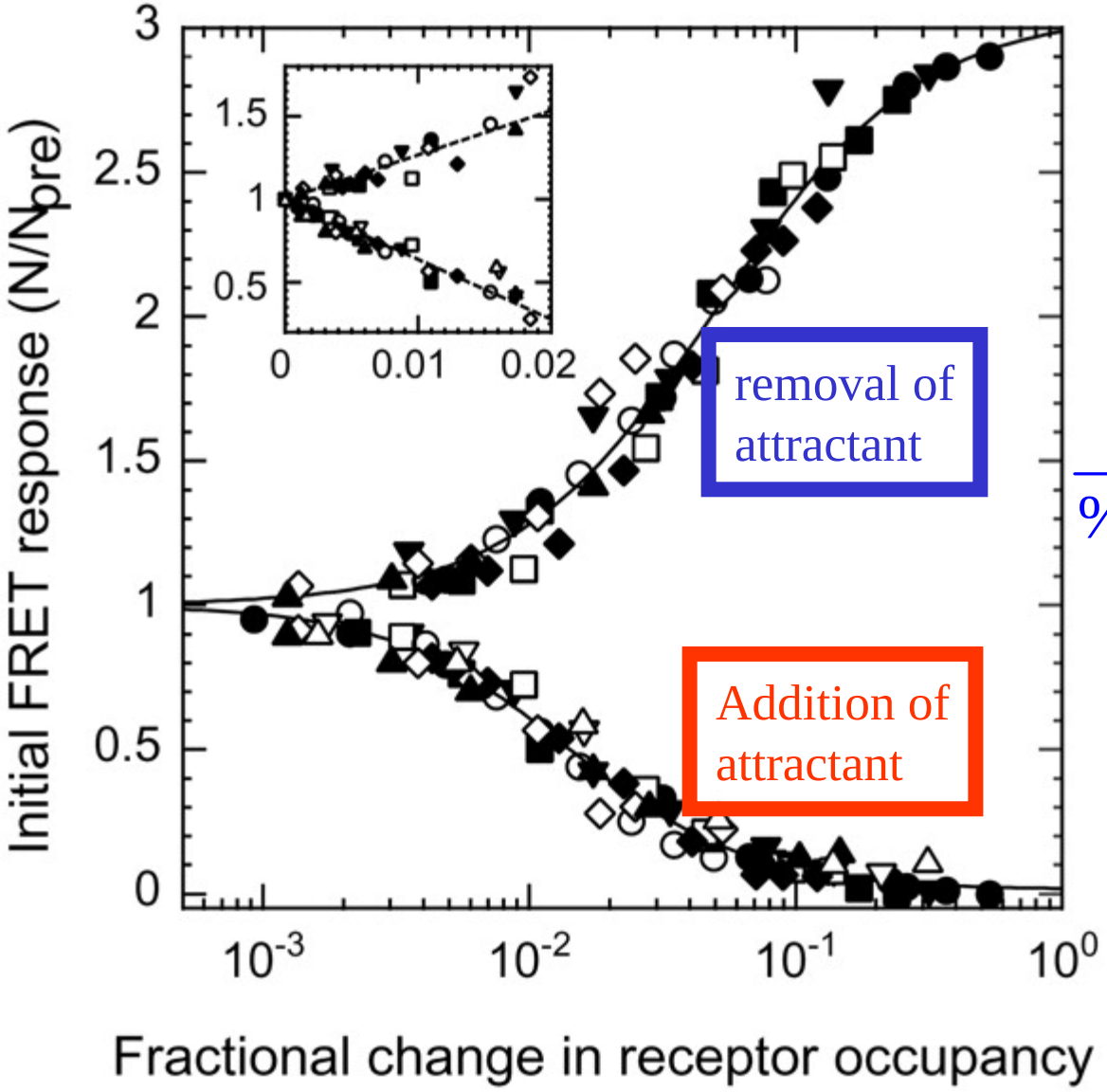
Experiment 1:

•Cells tracked ~6mm from the capillary with 1mM aspartate:
•Gradient: 0.02 μM/μm
•Mean concentration: 8μM
•Run length 10μm
•Fractional change in concentration: 2.5%
•Fractional change in receptor occupancy: 0.003
•Runs up the gradient increased in length by 30%

Experiment 2:

• Tethered cell
• Fractional change in receptor occupancy: 0.002
• Rotational bias 0.23

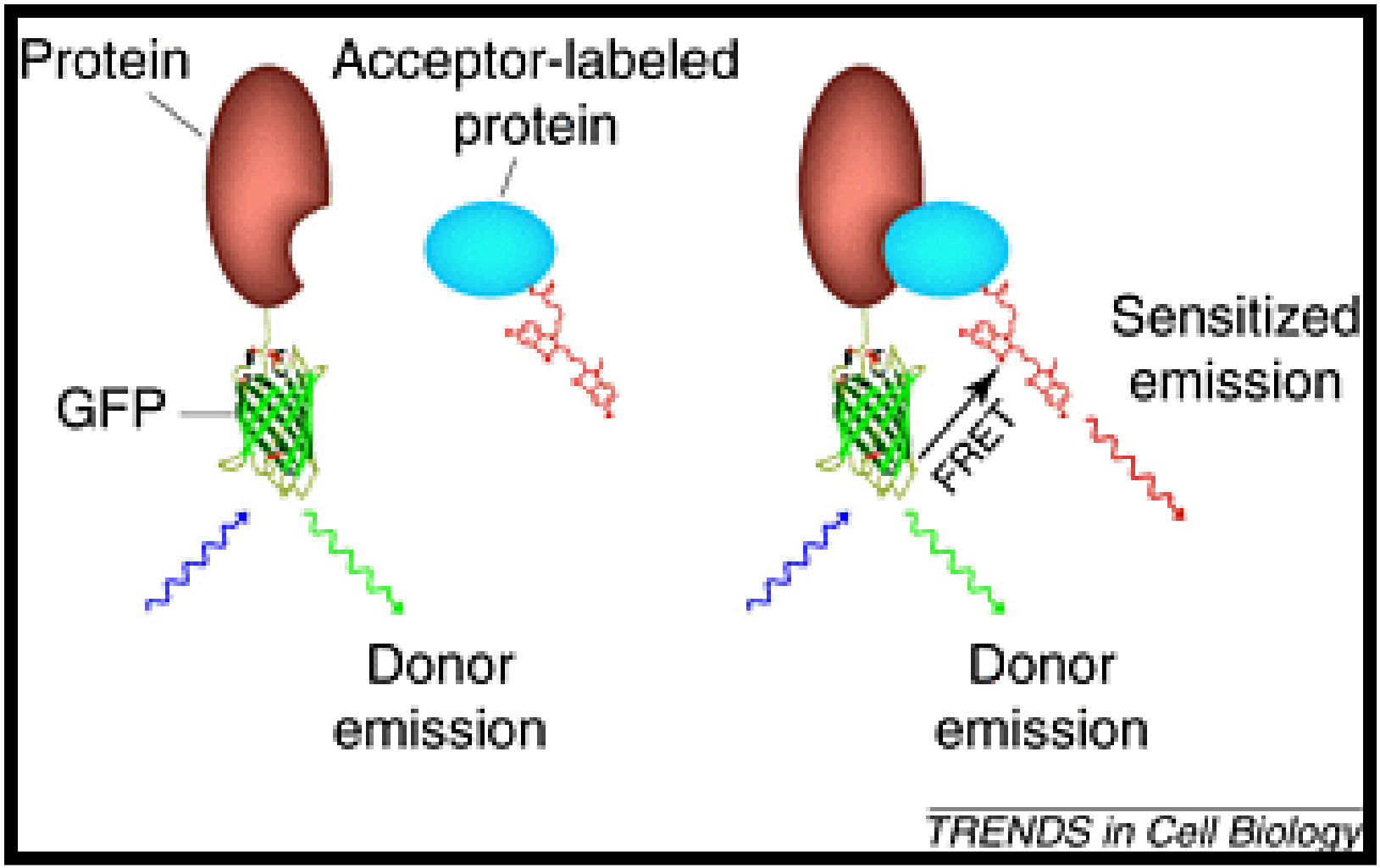
The gain is prodigious



$$\frac{\%\Delta CheY-P}{\%\Delta \text{Occupied receptors}} > 30$$

Experiment:
FRET for protein/protein Interactions

Berg & Sourjik, 2002

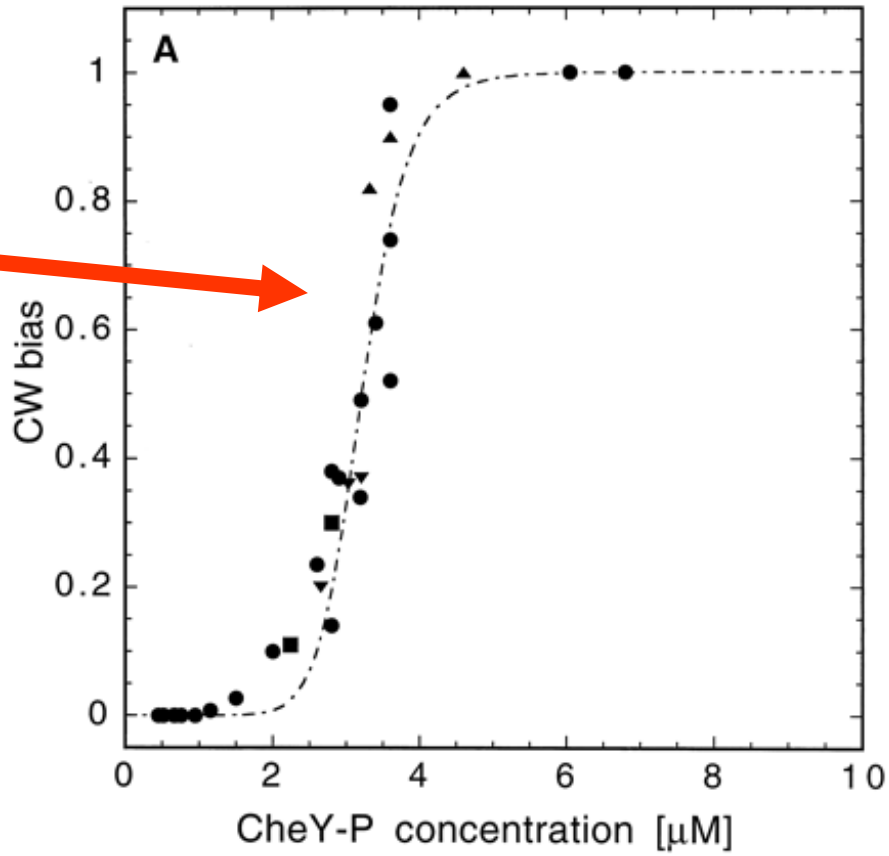

Protein
Acceptor-labeled protein
GFP
Donor emission
FRET
Sensitized emission
Donor emission
TRENDS in Cell Biology

What keeps the gain large
over the range of concentrations ?

How is it used?

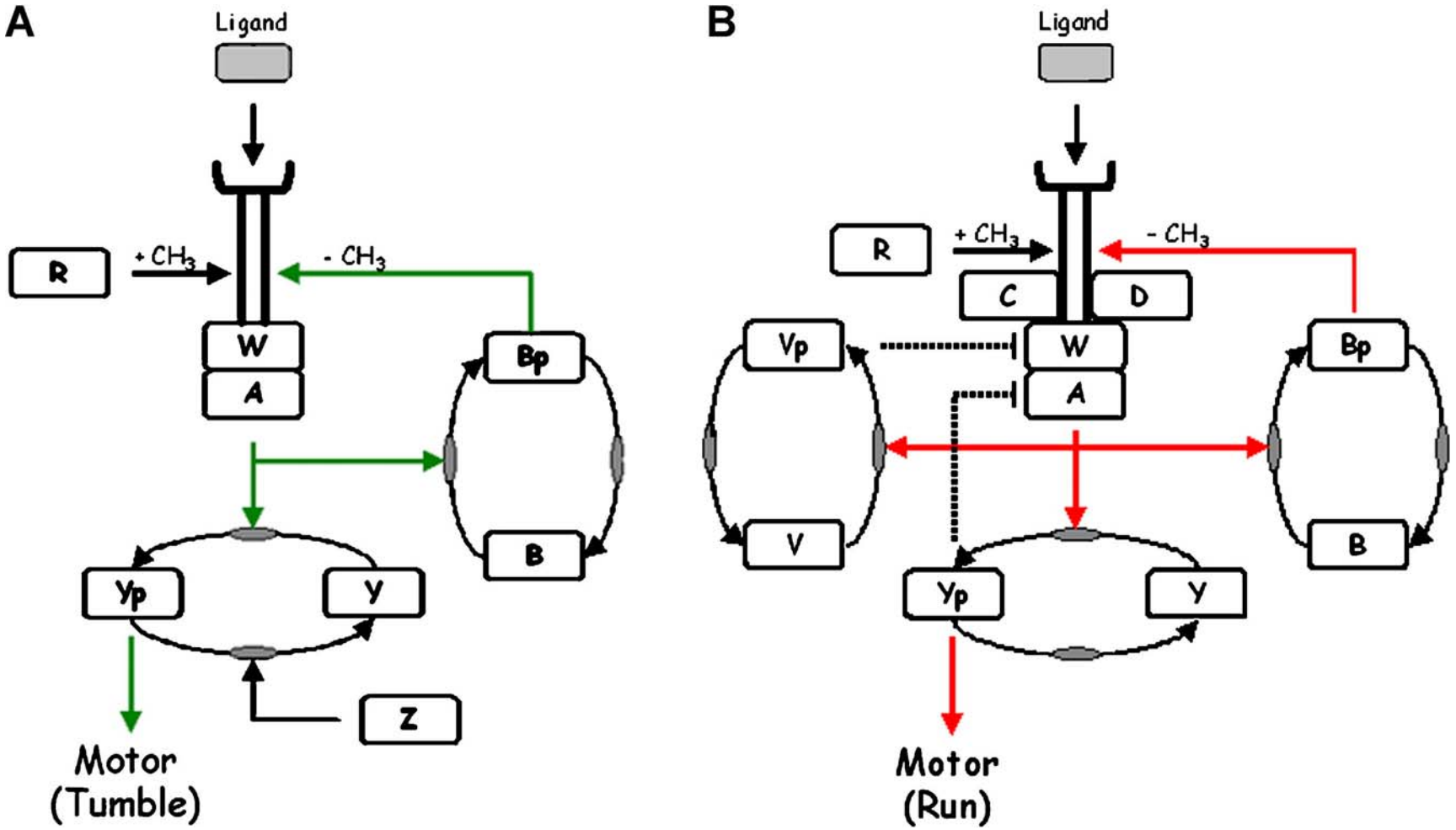
The motor appears to be ultransensitive (Cluzel, Surette, Leibler, 2000)

The Che-Yp in a fully adapted cell appears to be near the threshold



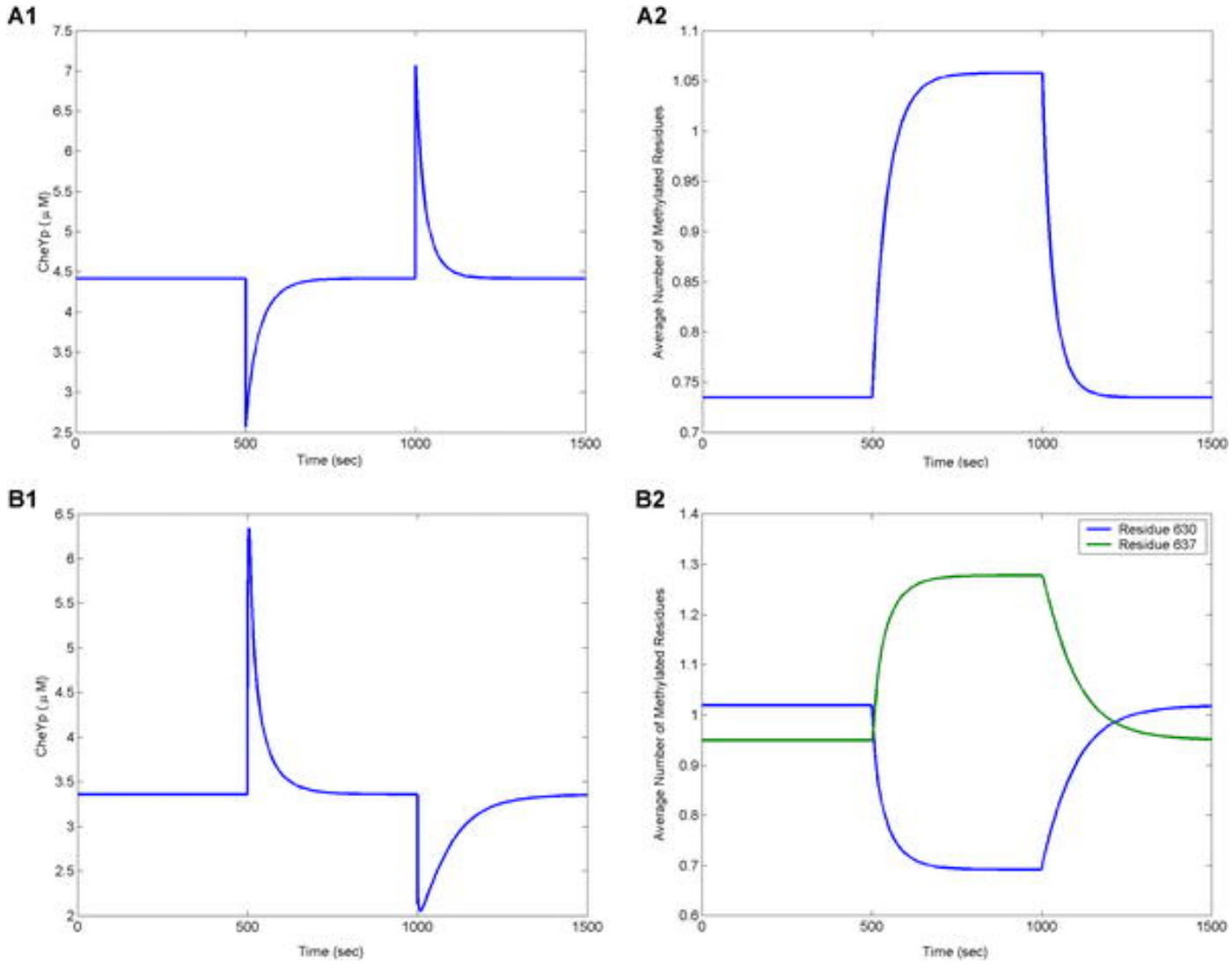
(Cluzel, Surette, Leibler, 2000)

Chemotaxis network in different species



Rao CV, Kirby JR, Arkin AP. Design and Diversity in Bacterial Chemotaxis: A Comparative Study in Escherichia coli and Bacillus subtilis. PLoS Biol. 2004 Feb;2(2):E49.

Both networks can robustly adapt



Rao CV, Kirby JR, Arkin AP. Design and Diversity in Bacterial Chemotaxis: A Comparative Study in Escherichia coli and Bacillus subtilis. PLoS Biol. 2004 Feb;2(2):E49.