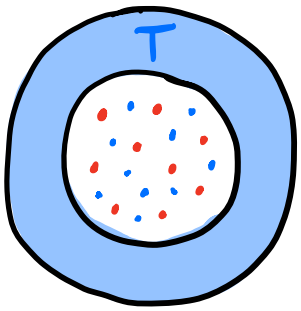
Announcements

1. Advance copy of notes on Canvas (Week 6)
2. New practice problem (Michaelis-Menten + competitive binding)

Supplemental Reading (chemotaxis)

1. Purcell (1977) "Life @ low Reynolds number"
2. Berg (1971) "How to track bacteria"
* 3. Berg & Brown (1972) "Chemotaxis in E.coli analyzed by three dimensional tracking"
4. Block et al (1982) "Impulse responses in bacterial chemotaxis"

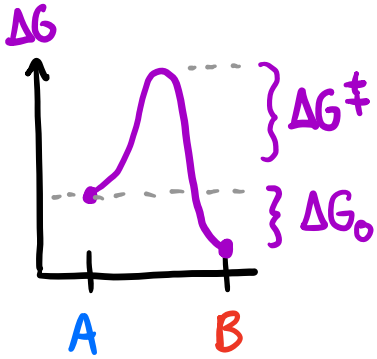
Last time: Rate eqns & dynamics inside the cell



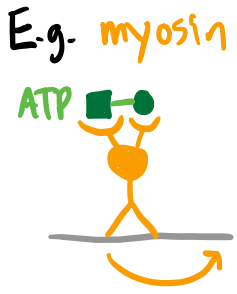
$$A \underset{k_{B\to A}}{\overset{k_{A\to B}}{\rightleftarrows}} B \iff \frac{d[B]}{dt} = k_{A\to B}[A] - k_{B\to A}[B]$$

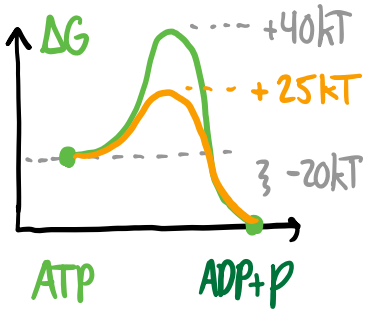
1. **Equilibrium:** $\frac{[B]}{[A]} = \frac{k_{A\to B}}{k_{B\to A}} = e^{-\Delta G_0/kT}$

2. **Arrhenius Law:** $k_{A\to B} = A \cdot e^{-\Delta G^{\ddagger}/kT}$



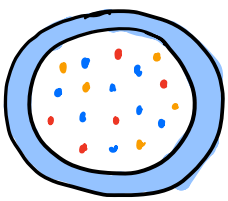
3. **Enzymes** speed up reactions by lowering **activation energy**





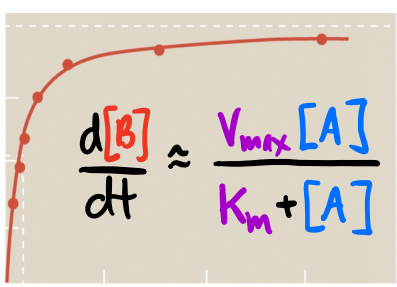
Note: saves **time**, but not **energy**!

4. **Michaelis-Menten Kinetics**



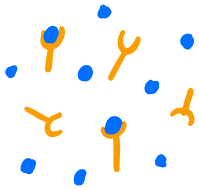
$$A + E \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} AE \overset{k_{cat}}{\rightarrow} B + E \Rightarrow$$

when $k_{on}[A] \gg k_{cat}$:

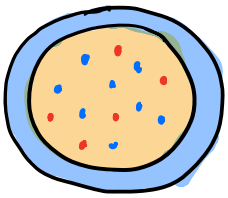


$$\frac{d[B]}{dt} \approx \frac{V_{max}[A]}{K_m + [A]}$$

w/ $V_{max} \equiv k_{cat}[E]_0$, $K_m \equiv \frac{k_{off} + k_{cat}}{k_{on}}$



$\Rightarrow$ Equivalent to "effective reaction"

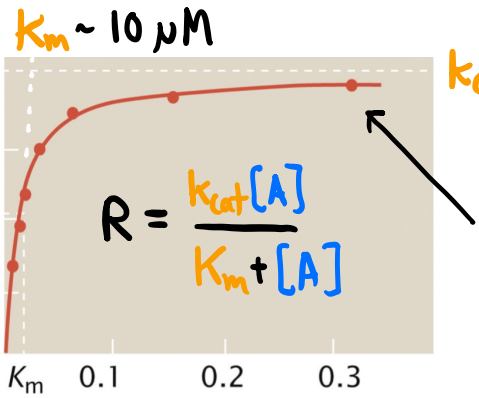


$A \xrightarrow{k_{eff}} B$ w/ $k_{eff}([E],[A]) \equiv \frac{k_{cat}[E]}{K_m + [A]}$

Today: (1) can measure k_{cat}, K_m in (bulk) experiments.

Rxns/enzyme/time:

$$R \equiv \frac{1}{[E]} \frac{d[B]}{dt}$$



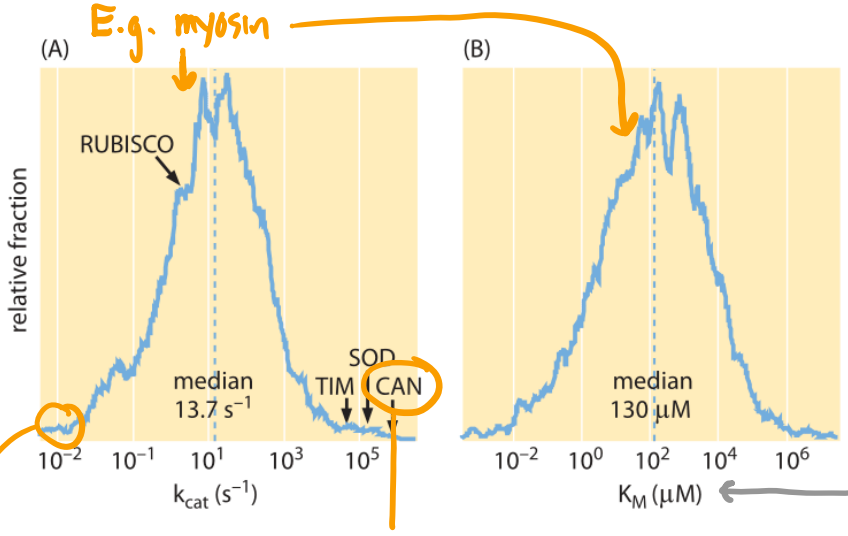
E.g. myosin in vitro

vs

$K_m \sim 0.01\,mM = 10\,\mu M$

$\Rightarrow$ Lecture 3: cellular [ATP] ~ 1mM $\Rightarrow$ operates in saturated regime

Question: How does this compare to other enzymes?



⇐ dist'n of k_{cat} & K_m across >1000 enzymes (Bar-Even et al 2011)

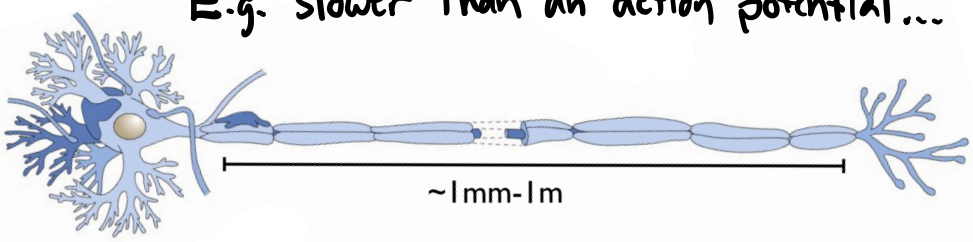
1μM ~ 1000 molecules / E.coli cell

restriction enzymes: $k_{cat} \sim 1\ min^{-1}$

carbonic anhydrase: $k_{cat} \sim 1\ \mu s^{-1}$

Lesson: huge range, but many enzymes "slow" ($k_{cat} \lesssim 100\ s^{-1}$)

E.g. slower than an action potential...

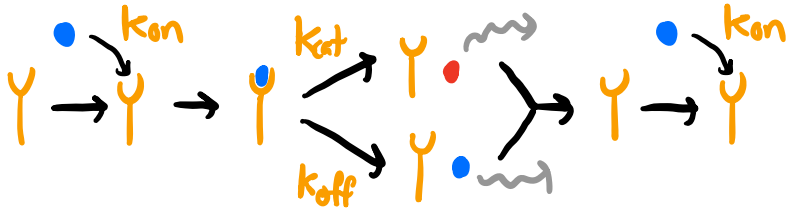


~ 1-10 ms

Question: is there an upper limit to enzyme efficiency?

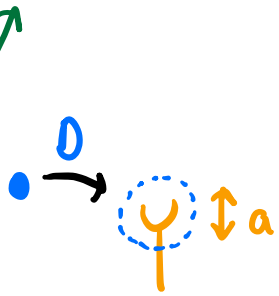
⇒ focus on $[A] \ll K_m$ (i.e. E finishes before next A arrives...)

Single enzyme:



⇒ from Michaelis-Menten Eq: $R \equiv \frac{1}{[E]}\frac{d[B]}{dt} \approx \frac{k_{cat}}{K_m} \cdot [A]$

⇒ But rate also limited by diffusion!



Lecture 5: $R \leq R_{max} \equiv 4\pi D a [A]$

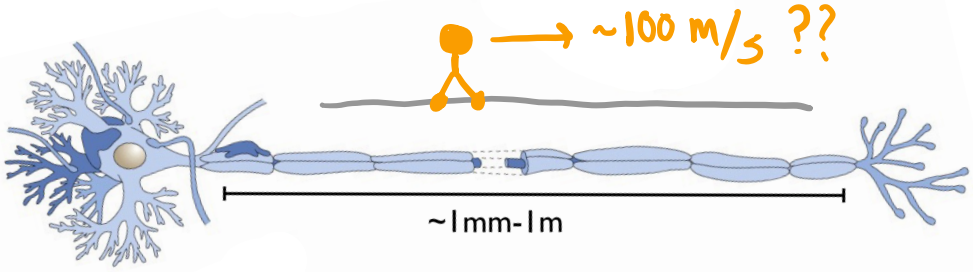
⇒ Diffusive speed limit to enzyme efficiency:

$$\frac{k_{cat}}{K_m} \leq 4\pi D a \sim 10\left(100\,\mu m^2/s\right)(1\,nm) \sim 10^9\ M^{-1}\cdot s^{-1}$$

Lecture 1: size of catalytic site (~ATP)

Lecture 4: Diffusion of ATP in cell ~$(10\mu m)^2/s$

E.g. imaginary example from Lecture 5: motor protein w/ speed of action potential??

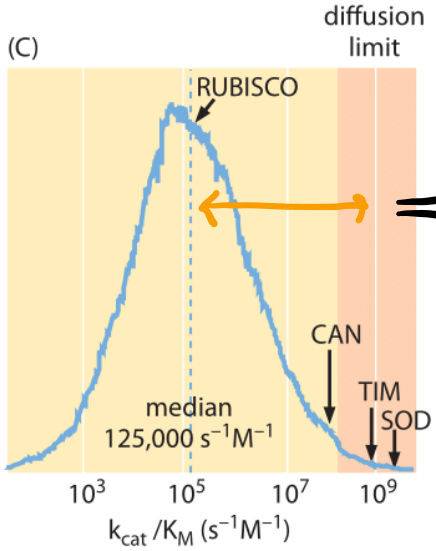


⇒ Needed to hydrolize ~5×10^{11} ATPs in 0.01 s ⇒ $R = 5\times10^{13}\ \frac{ATP}{s}$

⇒ But diffusive speed limit: $R \leq 10^9\ M^{-1}s^{-1}\cdot[ATP] \approx 10^6\ \frac{ATP}{s}$ ($[ATP] \approx 10^{-3}\ M$)

⇒ need 10^7-fold higher [ATP] to work! ✗

Question: How do real enzymes compare to diffusion limit?



⟹ most are >1000x lower than limit!

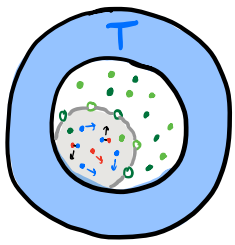
⇒ a few exceptions: "catalytically perfect enzymes"

Summary of rate eqns discussion:

Question: How do cells build costly molecules...

$A + B + ATP \rightleftarrows AB + ADP + P$ ✓

~~$ATP \rightleftarrows ADP + P$~~



... while preventing others from reaching equilibrium?

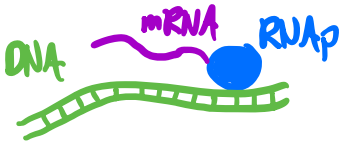
Lesson: cells choreograph rxns by modulating timescales! (using enzymes that lower activation energies)

E.g. $\frac{d[ATP]}{dt} = \underbrace{k_{off}^{spont}}_{10^{-3}\,s^{-1}}[ATP] + \underbrace{k_{AB}([E_{NB}])[A][B]}_{\text{e.g. } 100\,s^{-1}}[ATP] + \dots$

$\Rightarrow$ can speed up rxns in highly tunable way (via [E])

(i.e. slow spontaneous rxns are feature not bug!)

E.g. "gene regulation" (transcribing + translating more enzyme)



$\Rightarrow$


$\Rightarrow$ $\gamma\,\gamma\,\gamma\,[E]$

$\Rightarrow$ Relatively slow (Lecture 1: $\tau_{trans} \geq 10s$)

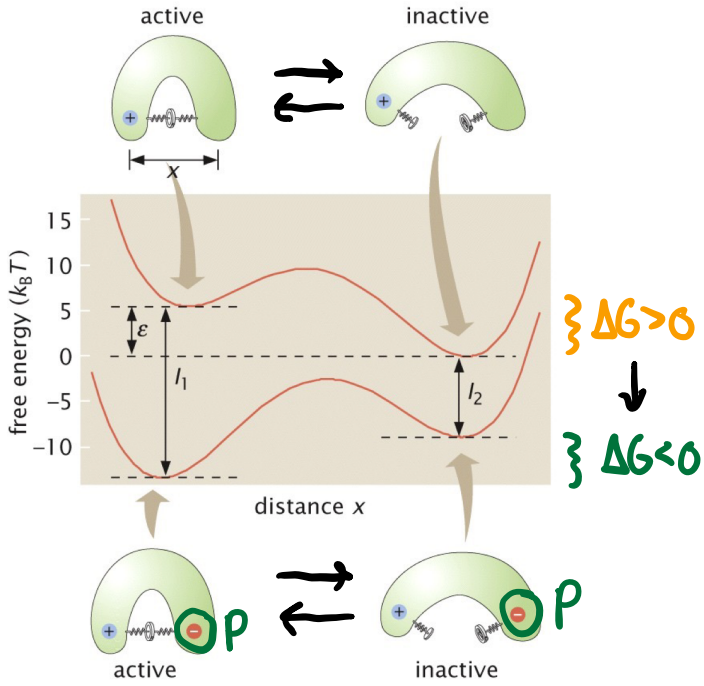
$\Rightarrow$ Faster alternative: "post-translational modification"

E.g. "phosphorylation": $E + ATP \rightarrow E\text{-}P + ADP$ (i.e. add phosphate)

$E_1\text{-}P + E_2 \rightarrow E_1 + E_2\text{-}P$ (transfer phosphate)

↑ catalyzed by other enzymes

Why is this useful?



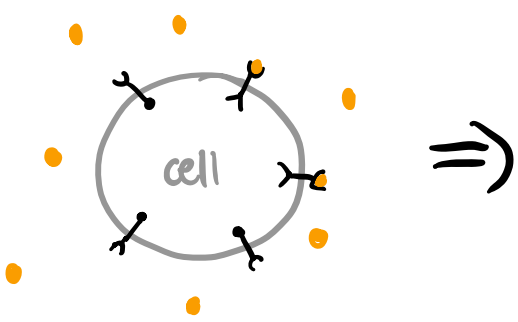
① can modify shape (& therefore function) of target enzyme

② fast & tunable (since catalyzed by other enzymes)

"kinase" E_k : $E + ATP \xrightarrow{k_{on}([E_k])} E\text{-}P + ADP$

"phosphotase" E_p : $E\text{-}P \xrightarrow{k_{off}([E_p])} E + P$

E.g. two component signal transduction systems in bacteria:



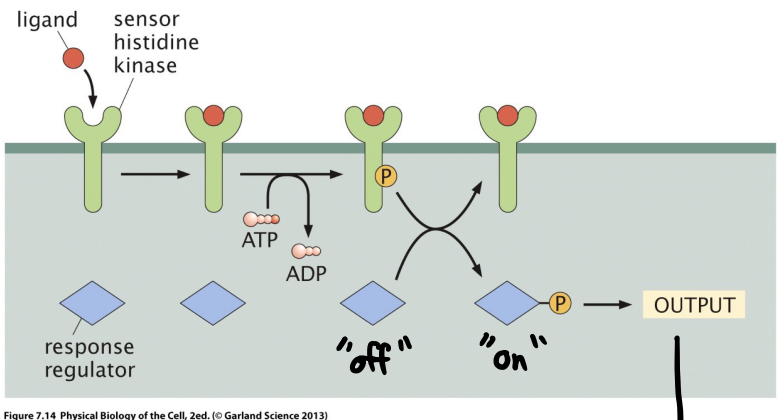


Figure 7.14 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

e.g. initiate transcription
catalyze reaction, ...

⇒ Common strategy! Two component systems in E. coli:

important for chemotaxis...

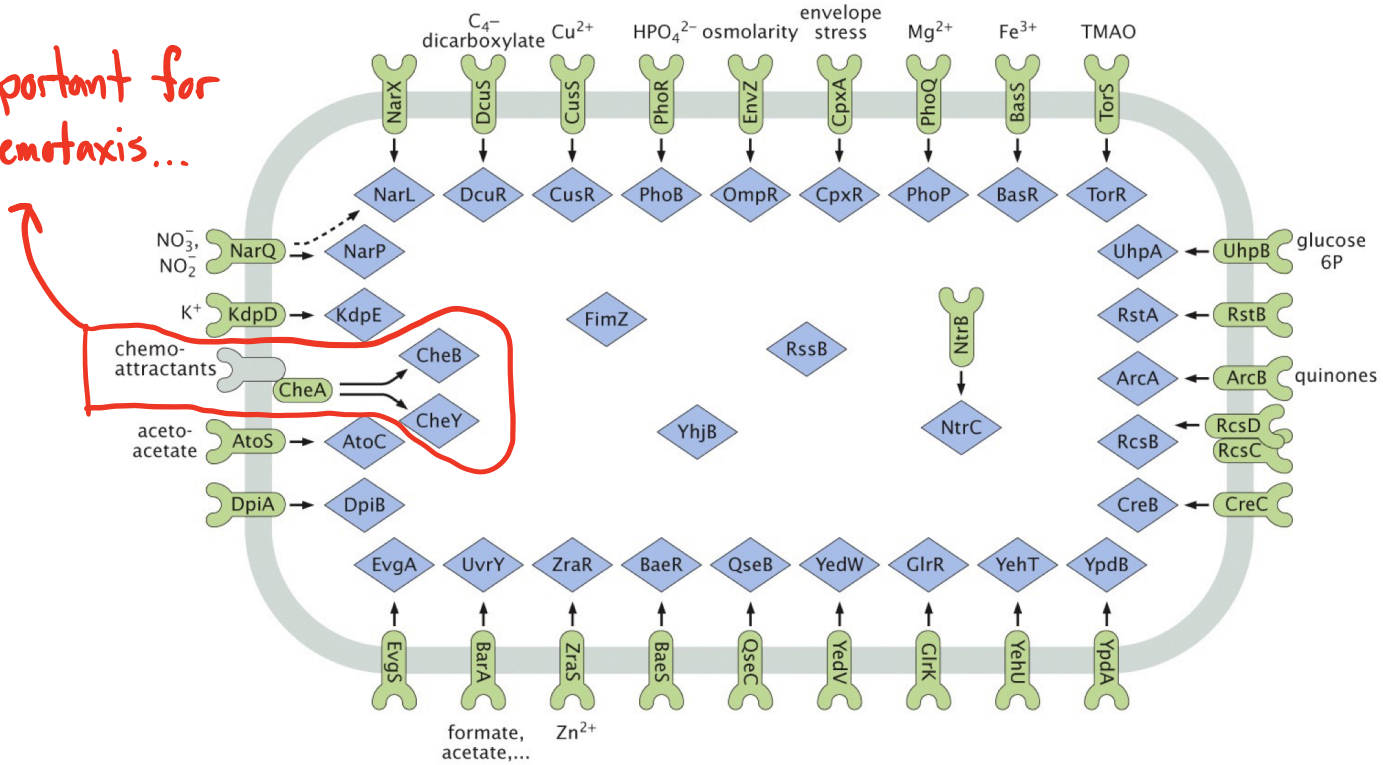


Figure 7.15 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Next: bacterial chemotaxis (today + next lecture)

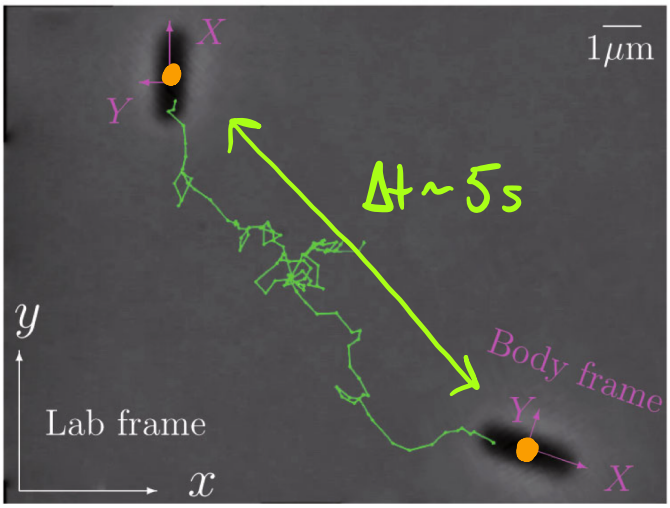
⇒ classic example of complex behavior in bacteria

⇒ brings together many concepts we have introduced so far...

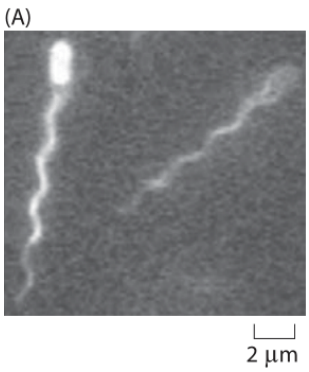
Recall Lecture 4:

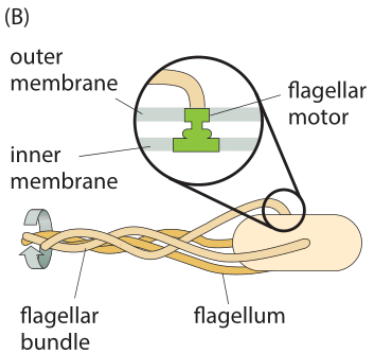
E.coli passively jostled around via diffusion

($D \sim 1\,\mu m^2/s$)

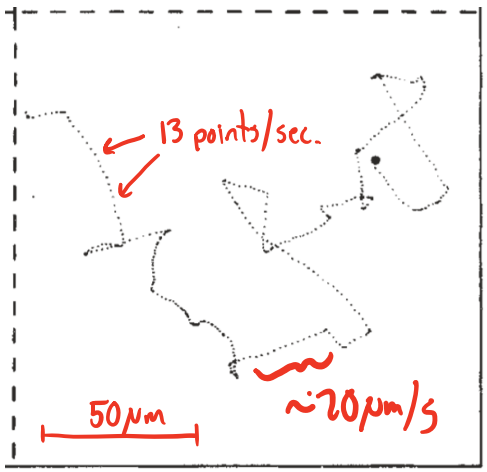


⇒ E.coli can also "swim"!





⇒



Berg & Brown (1972)

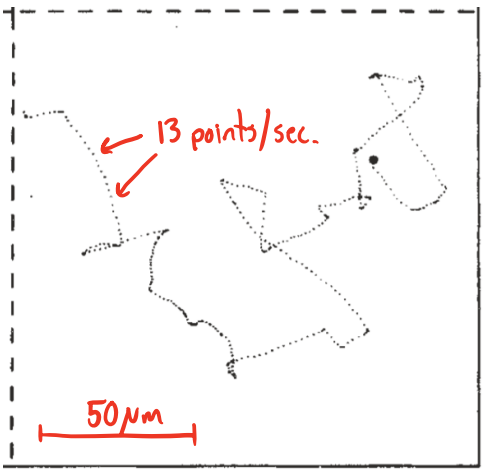
⇒ can travel ~ 20μm in ~1s! ($\gg \sqrt{Dt} \sim 1\mu m$)

⇒ requires constant input of energy ($\int v e^{-\frac{\gamma}{m}t} dt < 10^{-10}$ m)

⇒ Paths: "runs" interspersed w/ sudden changes in direction

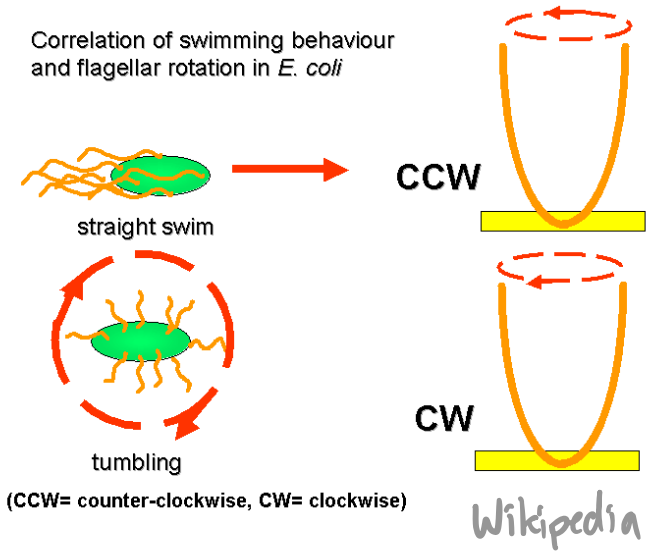
"tumbles"

Runs & tumbles arise from rotation of flagella:



(see video)

$\Leftrightarrow$

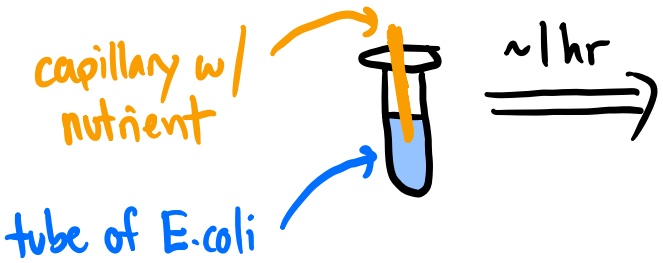


$\Rightarrow$ Net result: cell executes an (active) "random walk" on timescales $\gg$ 1s (typical run length)

$\Rightarrow$ is this good for anything??

One example: chemotaxis (Pfeffer, 1880s, Adler 1960s, + others)

classic assay:



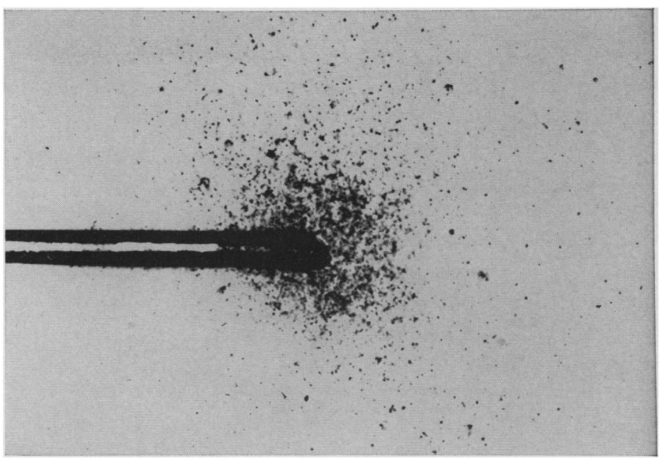


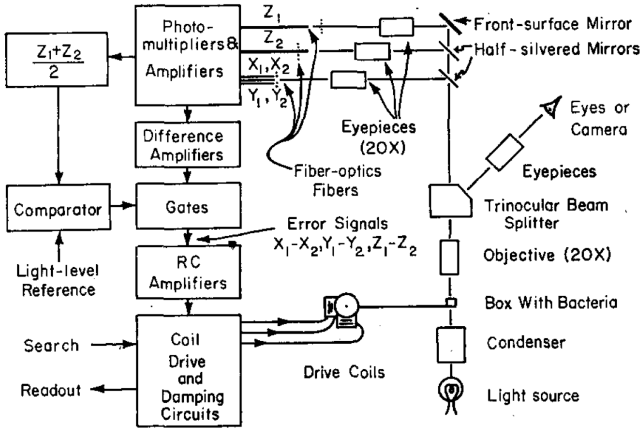
Fig. 1. Photomicrograph showing attraction of *Escherichia coli* bacteria to aspartate. The capillary tube (diameter, ~ 25 microns) contained aspartate at a concentration of $2 \times 10^{-3} M$. [Photomicrograph by Scott W. Ramsey; dark-field photography]

⇒ cells manage to swim toward nutrient ("chemotaxis")

⇒ How do they do this?? (& why?)

Howard Berg: must be able to track individual cells over long distances @ high resolution...

⇒ built 3D tracking microscope (1970)



Berg & Brown (1972)

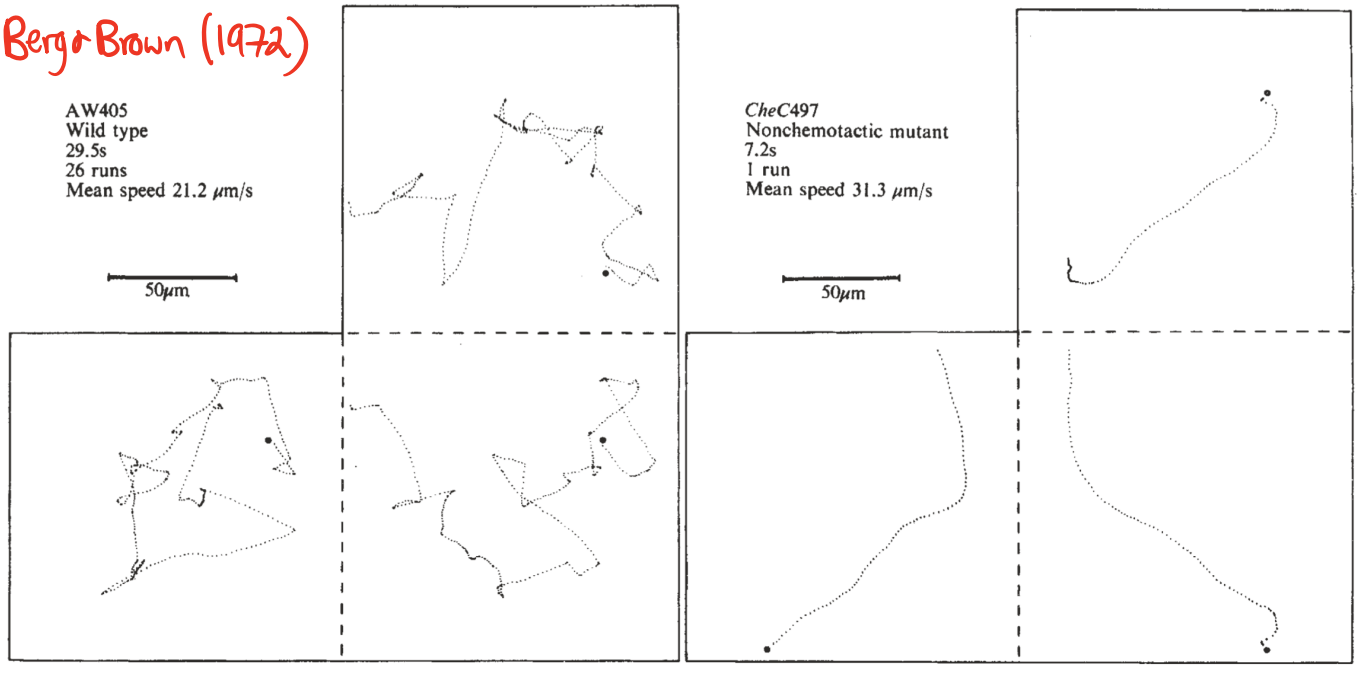
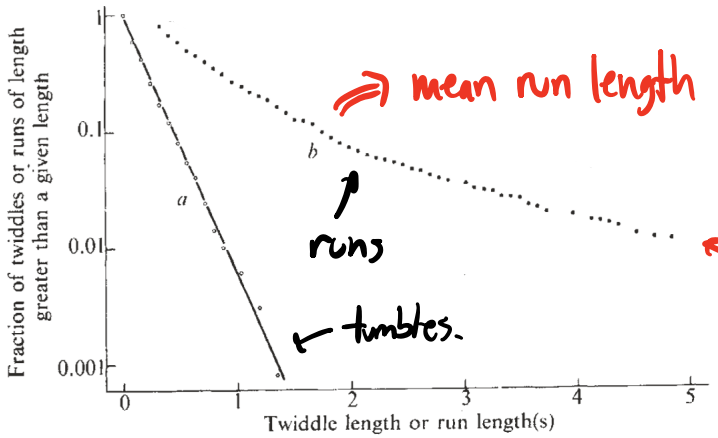


Fig. 1 Digital plots of the displacement of a wild type bacterium, AW405, and a generally nonchemotactic mutant, *cheC* 497, at the rate of 12.6 words (data points) per second. Tracking began at the points indicated by the large dots. The plots are planar projections of three-

⇒ First clue: tumbles important for chemotaxis?

⇒ 2nd clue: data provided highly quantitative statistics of runs & tumbles:



⇒ mean run length = 0.83 s

runs

tumbles.

← aggregated across ~600 runs!

SE = SD/√600

can ask how dist'n changes across conditions:

Table 2 Run-twiddle Analysis of the Wild Type Swimming in Gradients

Attractant	Serine		Aspartate	
	Control	Gradient	Control	Gradient
Number of bacteria tracked	11	34	23	24
Total tracking time (min)	7.1	14.8	11.1	11.0
Mean run length (s)	0.83 ± 0.88	1.67 ± 2.56	0.83 ± 0.90	0.90 ± 1.56
Mean concentration of attractant (μM)	0	9.5 ± 2.7	10	8.4 ± 2.0
Mean distance from mouth of capillary (μM)	—	577 ± 112	—	644 ± 88
Mean value of $(\partial C/\partial r)/C$ (mm^{-1})	—	2.5 ± 0.4	—	2.4 ± 0.4

⇒ systematically different run lengths in gradient!

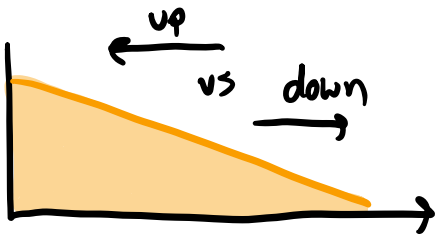
Final clue: run length correlated w/ direction of gradient

Table 3 Analysis of Runs which Move the Bacteria Up the Gradient or Down the Gradient

Attractant	Serine	Serine	Aspartate	Aspartate
Net displacement of runs	Up	Down	Up	Down
Mean concentration (μM)	10.0±2.8	9.2±2.6	8.8±1.9	8.1±1.9
Mean run length (s)	2.19±3.43	1.40±1.88	1.07±1.80	0.80±1.38
Mean run length expected from the control run length (Table 2) and the concentration dependence (Fig. 5) (s)	1.48	1.45	0.82	0.82

The runs of the gradient experiments (Table 2) divided into two subsets according to whether the net displacement of a run is toward or away from the mouth of the capillary (up-gradient or down-gradient). The mean speed was only slightly larger for runs up the gradient than for runs down the gradient (2% for serine, 7% for aspartate).

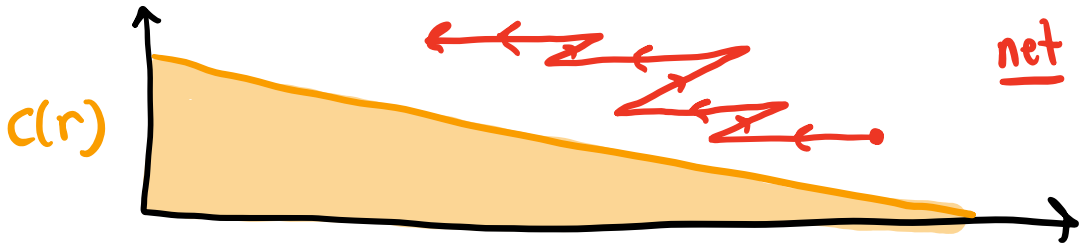
SD



(no correlation w/ tumbles)

⇒ chemotaxis = "biased random walk" up conc. gradient

(i.e. 2 steps forward, 1 step back)



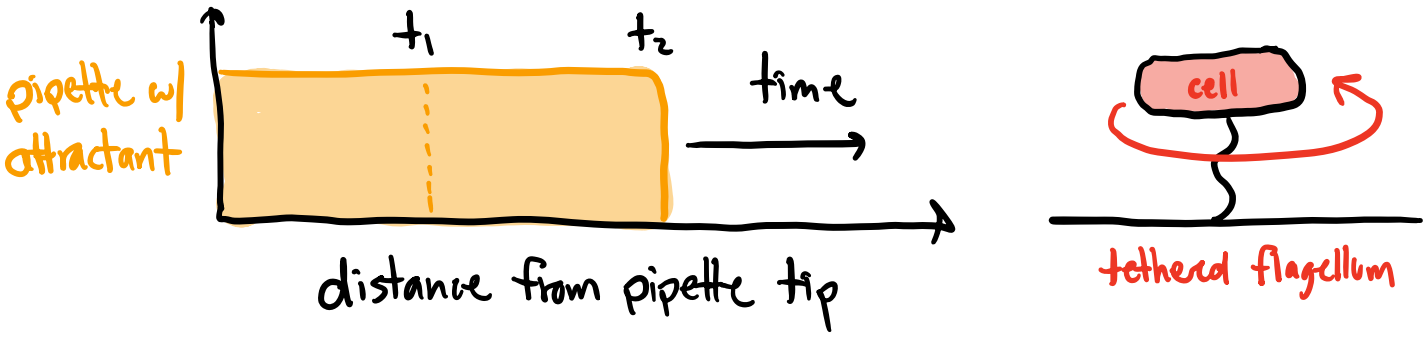
net speed ~ 1 μm/s

~ 3mm/hr.

Distance from mouth of capillary

⇒ cells must modulate tumble rate in response to measured gradients...

⇒ Further proof: tethering experiments (Block et al 1983)



Measured response:

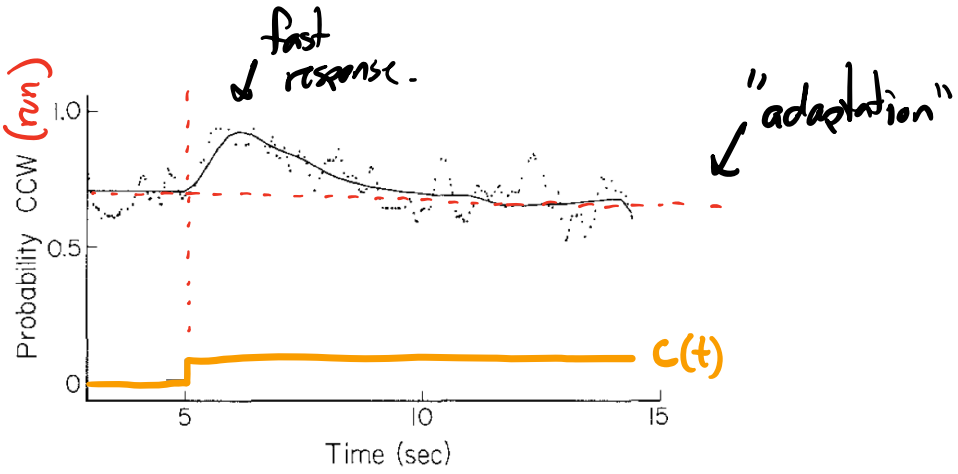


Figure 4. Small-Step Response of Wild-Type Cells

Next: how do cells measure gradients & implement adaptation w/ simple molecular toolbox?

Supplemental Reading (chemotaxis)

1. Purcell (1977) "Life @ low Reynolds number"
2. Berg (1971) "How to track bacteria"
3. Berg & Brown (1972) "Chemotaxis in E.coli analyzed by three dimensional tracking"
4. Block et al (1982) "Impulse responses in bacterial chemotaxis"