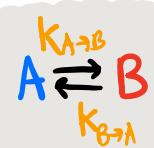
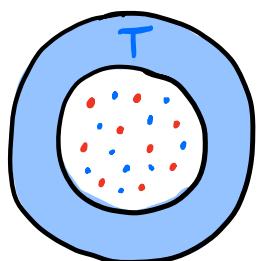


Announcements

- ① Advance copy of notes on Canvas (Week 6)
- ② New practice problem (Michaelis-Menten + competitive binding)
- ③ Week 6 reflections due Sat
- ④ Final project details coming soon (final paper, due March 24)

Last time: Rate eqns + dynamics inside the cell



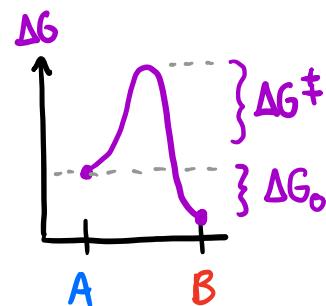
\Leftrightarrow

$$\frac{d[B]}{dt} = k_{A \rightarrow B}[A] - k_{B \rightarrow A}[B]$$

①

Equilibrium:

$$\frac{[B]}{[A]} = \frac{k_{A \rightarrow B}}{k_{B \rightarrow A}} = e^{-\Delta G_0/kT}$$

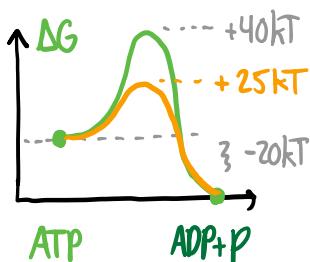


②

$$\text{Arrhenius Law: } k_{A \rightarrow B} = A \cdot e^{-\Delta G^\ddagger/kT}$$

③

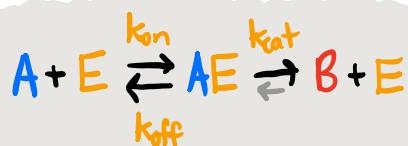
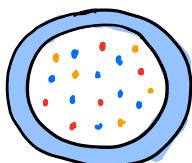
Enzymes speed up reactions by lowering activation energy



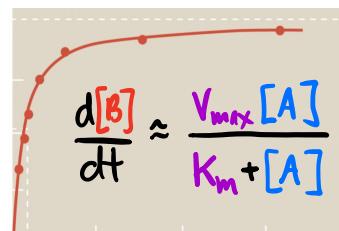
Note: Saves time, but not energy!

④

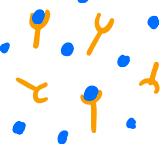
Michaelis-Menten Kinetics



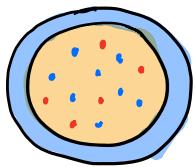
\Rightarrow



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

$$\omega | V_{\max} \equiv K_{cat}[E]_0, \quad K_m \equiv \frac{K_{off} + K_{cat}}{K_{on}}$$


\Rightarrow Equivalent to "effective reaction"



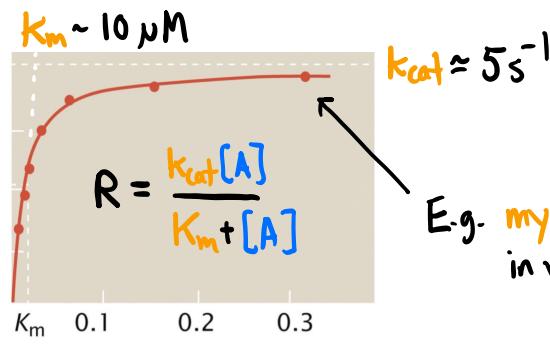
$\omega |$

$$k_{eff}([E], [A]) \equiv \frac{K_{cat}[E]}{K_m + [A]}$$

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

Rxns/enzyme/time:

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



E.g. myosin
in vitro



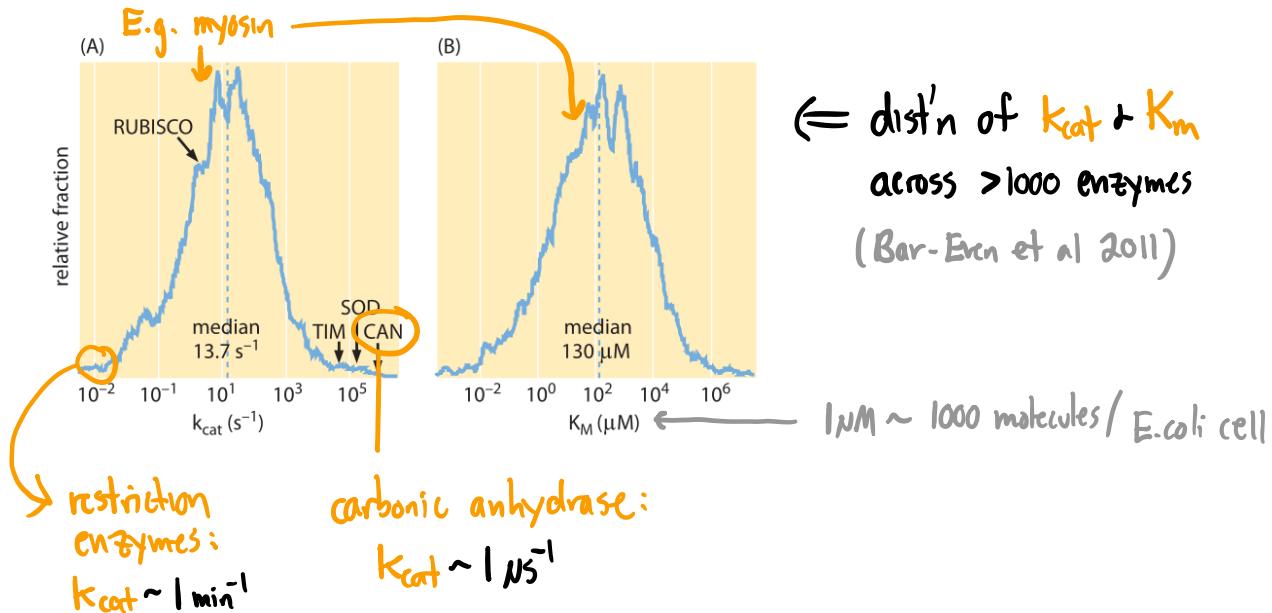
vs

$$[A] (\text{mM})$$

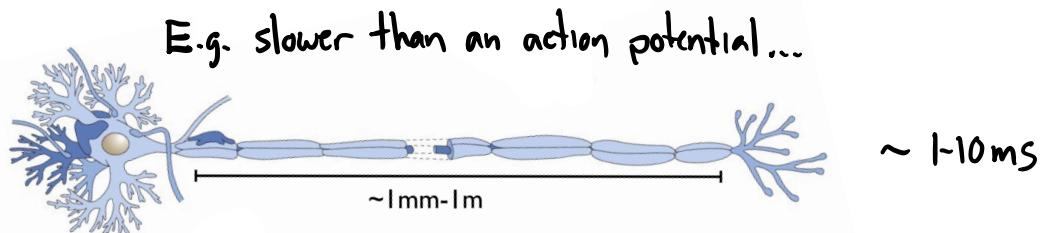
$$K_m > 0.01 \text{ mM} = 10 \text{ nM}$$

\Rightarrow Lecture 3: cellular $[ATP] \sim 1 \text{ mM} \Rightarrow$ operates in saturated regime

Question: How does this compare to other enzymes?

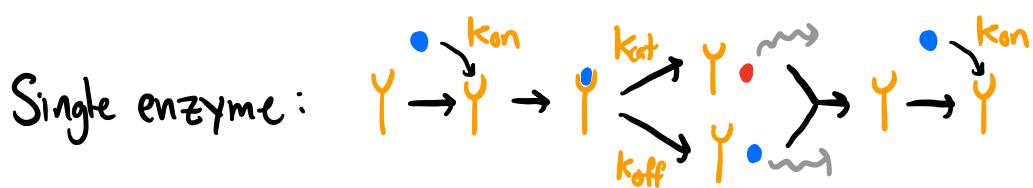


Lesson: huge range, but many enzymes "slow" ($k_{cat} \leq 100 \text{ s}^{-1}$)



Question: is there an upper limit to enzyme efficiency?

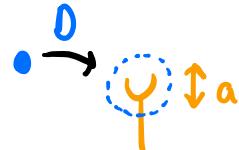
\Rightarrow focus on $[A] \ll K_m$ (i.e. E finishes before next A arrives...)



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

\Rightarrow But rate also limited by diffusion!

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



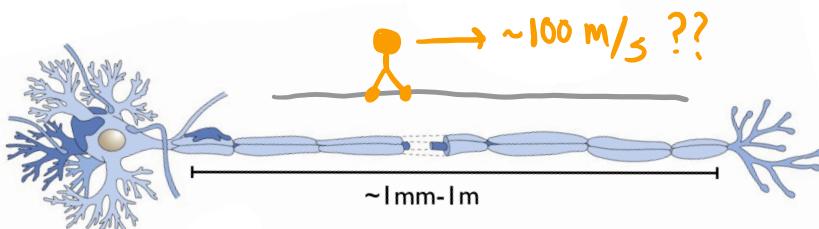
\Rightarrow Diffusive speed limit to enzyme efficiency:

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

\downarrow Lecture 1: size of catalytic site (\sim ATP)

\downarrow Lecture 4: Diffusion of ATP in cell $\sim (10\mu\text{m})^2/\text{s}$

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

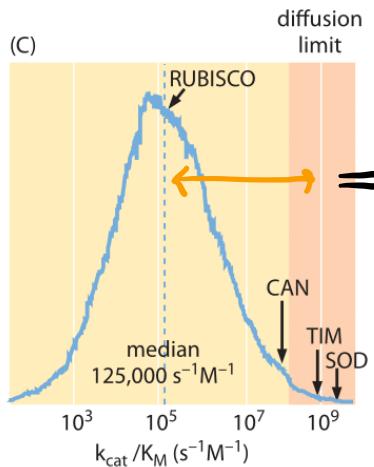


\Rightarrow Needed to hydrolyze $\sim 5 \times 10^{11}$ ATPs in 0.01 s $\Rightarrow R = 5 \times 10^{13} \frac{\text{ATP}}{\text{s}}$

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

\Rightarrow need 10^7 -fold higher $[\text{ATP}]$ to work! \times

Question: How do real enzymes compare to diffusion limit?

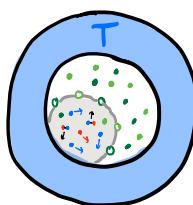


most are $>1000\times$ lower than limit!

\Rightarrow a few exceptions: "catalytically perfect enzymes"

Summary of rate eqns discussion:

Question: How do cells build costly molecules...



... while preventing others from reaching equilibrium?

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

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

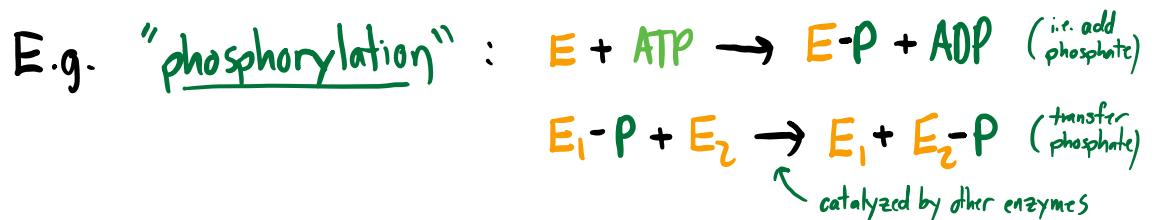
\Rightarrow can speed up rxns in highly tunable way (via $[E]$)
 (i.e. slow spontaneous rxns are feature not bug!)

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



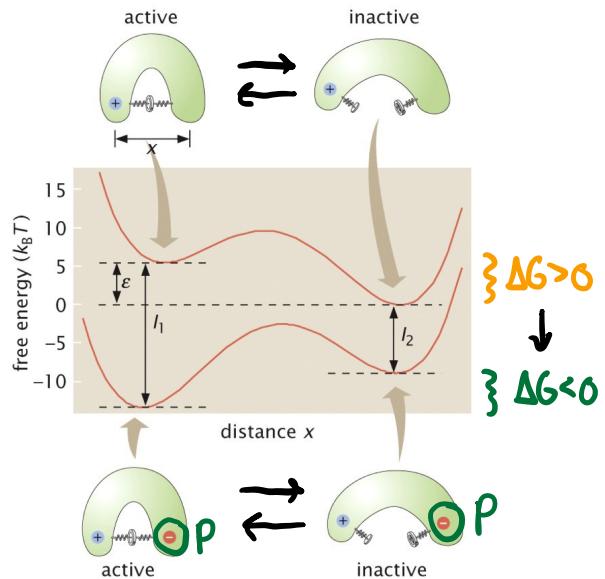
\Rightarrow Relatively slow (Lecture 1: $\tau_{\text{on}} \geq 10s$)

\Rightarrow Faster alternative: "post-translational modification"

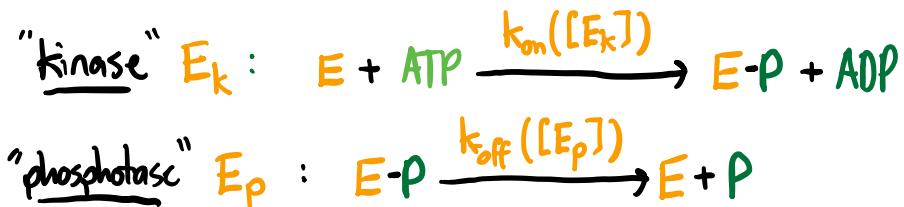


Why is this useful?

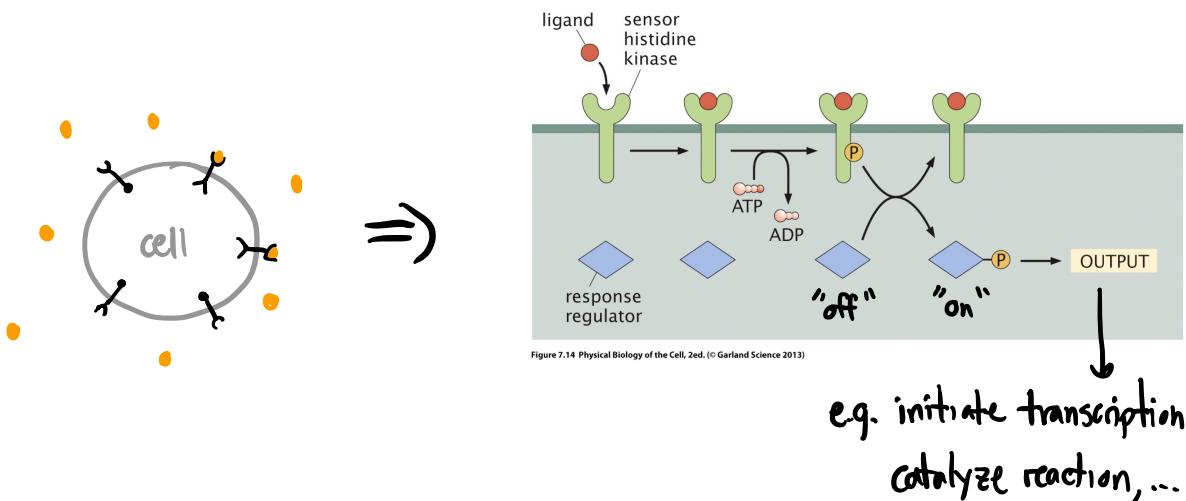
- ① can modify shape (and therefore function) of target enzyme



- ② fast & tunable (since catalyzed by other enzymes)



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



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

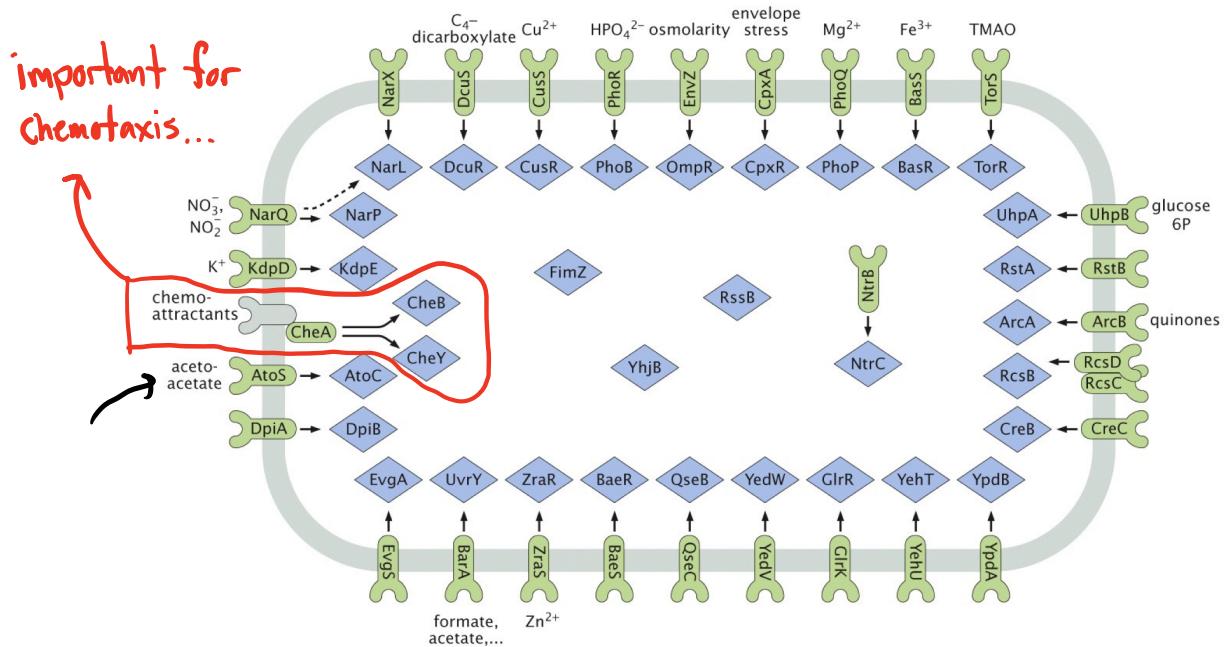


Figure 7.15 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Next: bacterial chemotaxis (today & next lecture)

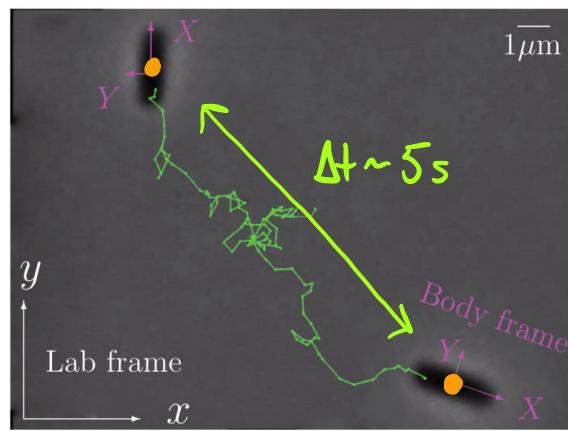
⇒ classic example of complex behavior in bacteria

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

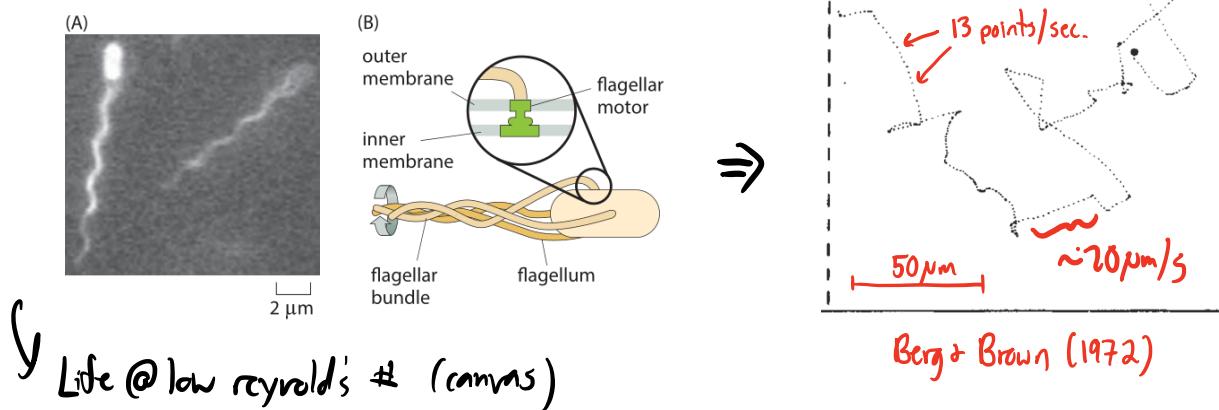
Recall Lecture 4:

E.coli passively jostled around via diffusion

$$(D \sim 1 \mu\text{m}^2/\text{s})$$



⇒ E.coli can also "swim"!

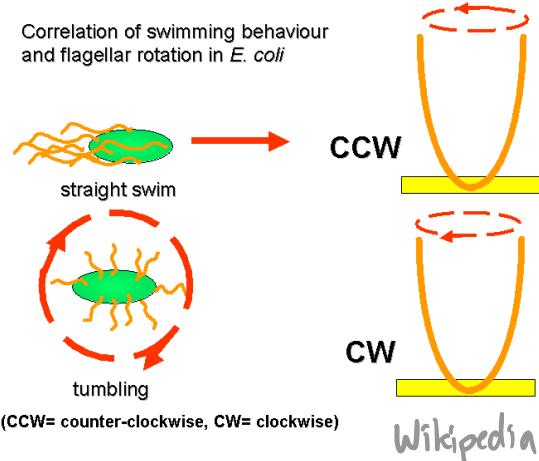
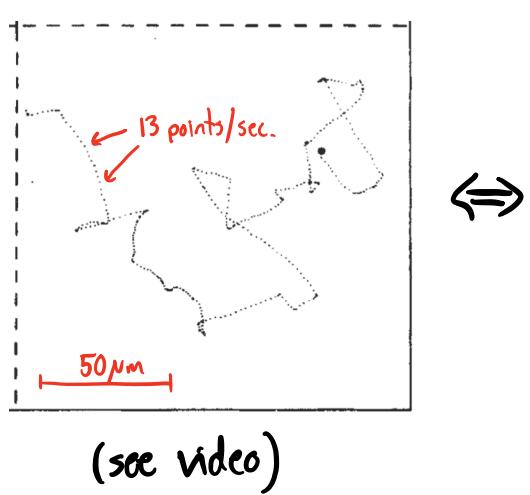


⇒ can travel $\sim 20 \mu\text{m}$ in $\sim 1\text{s}$! ($\gg \sqrt{Dt} \sim 1 \mu\text{m}$)

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

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

Runs & tumbles arise from rotation of flagella:



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

⇒ is this good for anything??

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

classic assay:

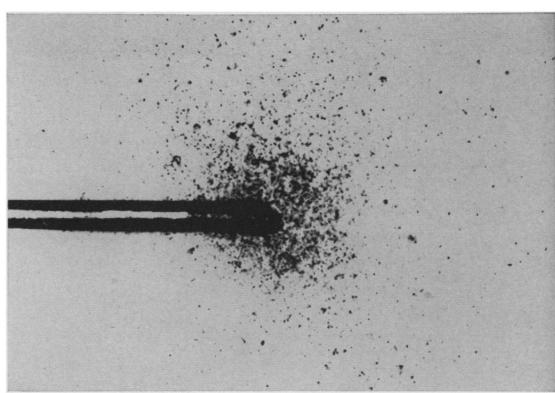
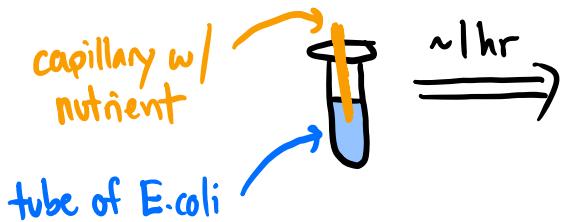


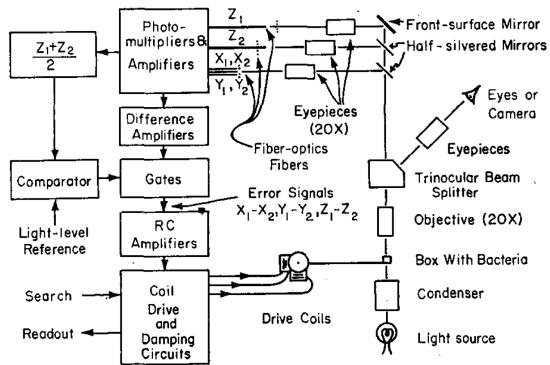
Fig. 1. Photomicrograph showing attraction of *Escherichia coli* bacteria to aspartate. The capillary tube (diameter, ~ 25 microns) contained aspartate at a concentration of $2 \times 10^{-5} M$. [Photomicrograph by Scott W. Ramsey; dark-field photography]

\Rightarrow cells manage to swim toward nutrient ("chemotaxis")

\Rightarrow How do they do this?? (+ why?)

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

\Rightarrow built 3D tracking microscope (1970)



Berg & Brown (1973)

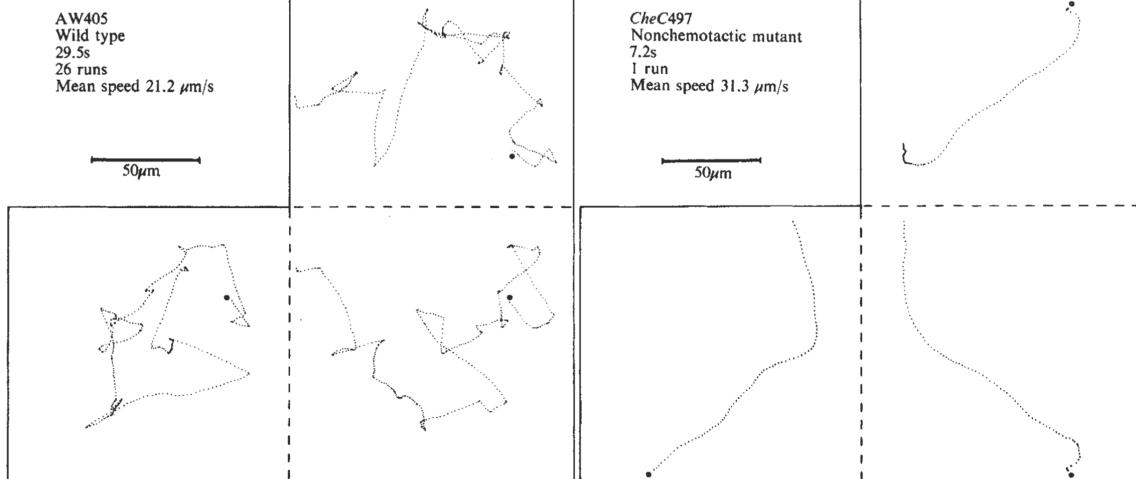
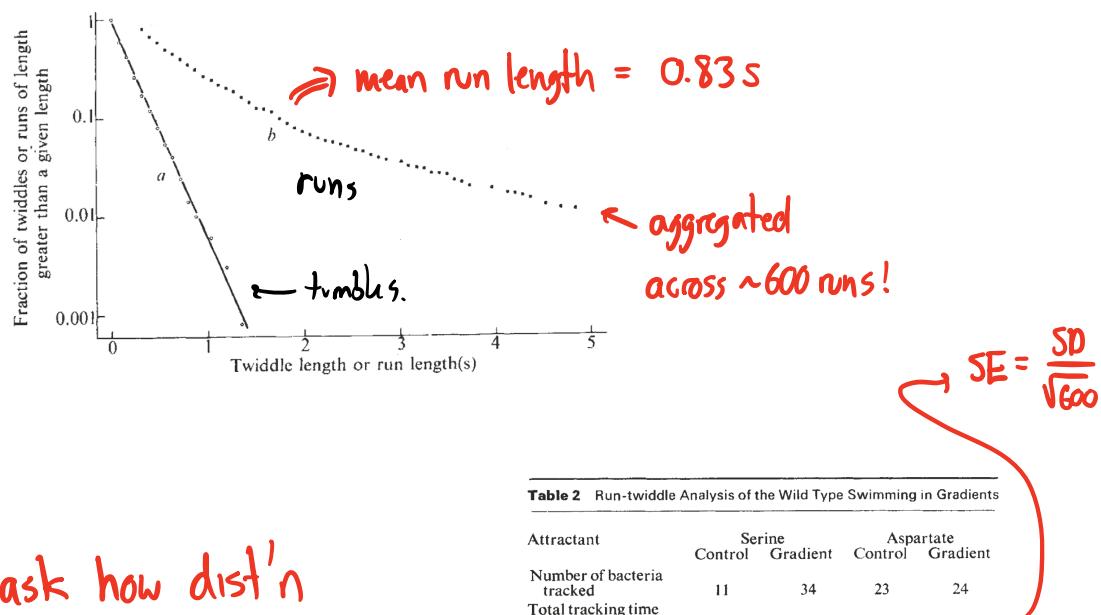


Fig. 1 Digital plots of the displacement of a wild type bacterium, AW405, and a generally nonchemotactic mutant, *cheC* 497, at the rate of 12.6 words (data points) per second. Tracking began at the points indicated by the large dots. The plots are planar projections of three-

\Rightarrow First clue: tumbles important for chemotaxis?

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

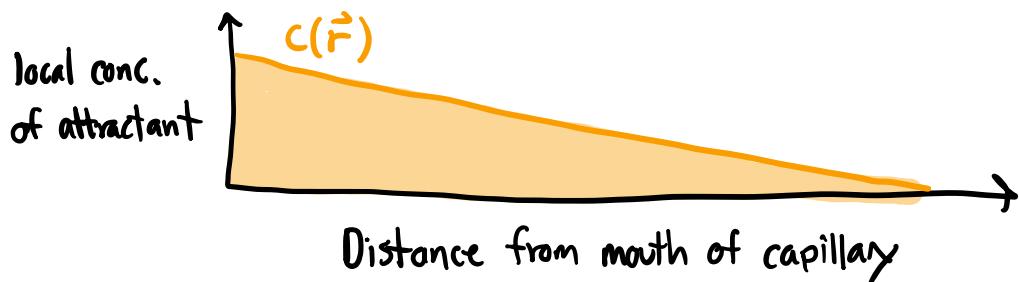


can ask how dist'n
changes across conditions:

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 $(\partial C/\partial r)/C (\text{mm}^{-1})$	—	2.5 ± 0.4	—	2.4 ± 0.4

\Rightarrow systematically different run lengths in gradient!

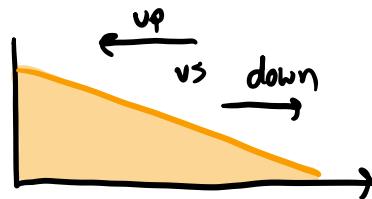


Final clue: run length correlated w/ direction of gradient

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

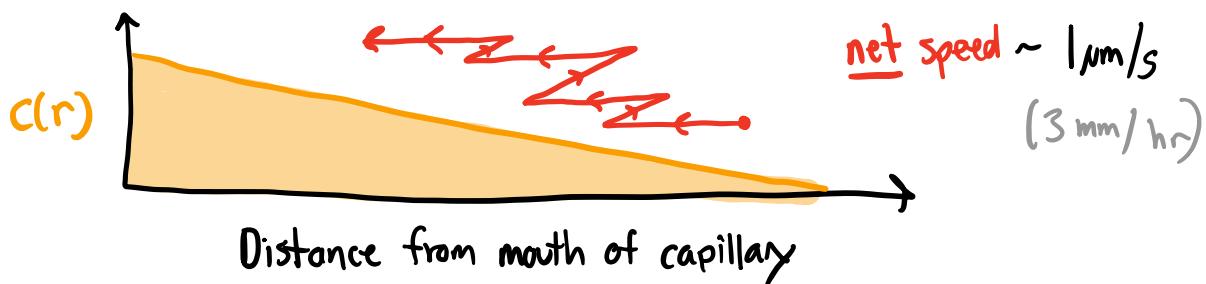
Attractant	Serine Net displacement of runs Up	Serine Down	Aspartate Up	Aspartate Down
Mean concentration (μM)	10.0 ± 2.8	9.2 ± 2.6	8.8 ± 1.9	8.1 ± 1.9
Mean run length (s)	2.19 ± 3.43	1.40 ± 1.88	1.07 ± 1.80	0.80 ± 1.38
Mean run length expected from the control run length (Table 2) and the concentration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



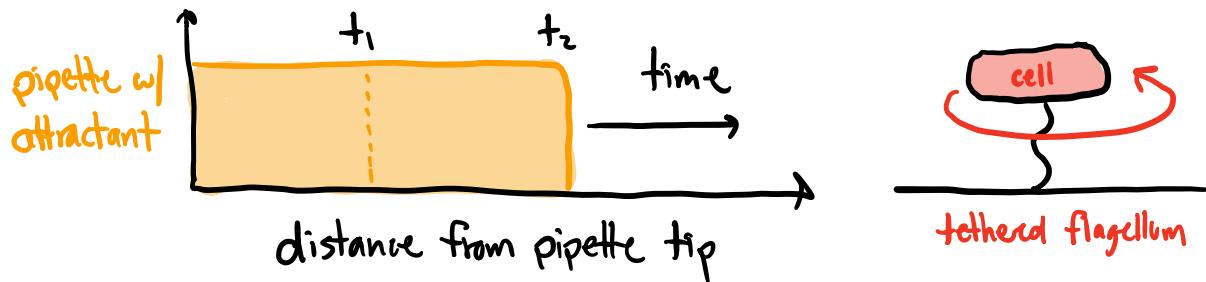
(no correlation w/ tumbles)

\Rightarrow chemotaxis = "biased random walk" up conc. gradient
(i.e. 2 steps forward, 1 step back)



\Rightarrow cells must modulate tumble rate
in response to measured gradients ...

\Rightarrow Further proof: tethering experiments (Block et al 1983)



Measured response:

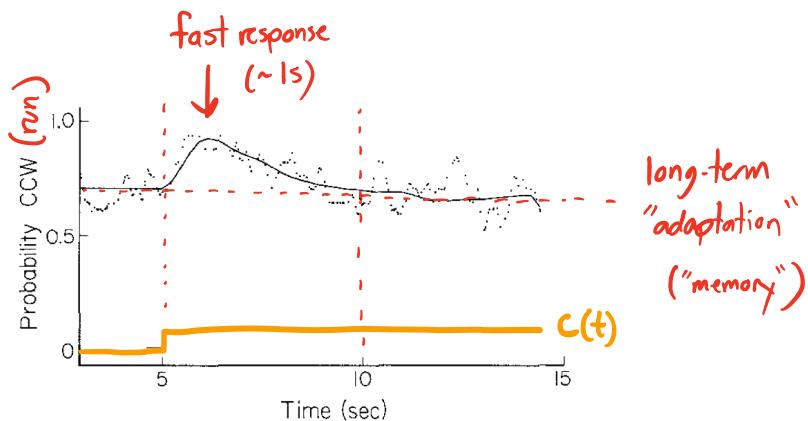


Figure 4. Small-Step Response of Wild-Type Cells

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