

Announcements:

- ① advance copy of notes in Week 7 folder
- ② Week 7 reflections due Sat.
- ③ Answers to practice problem 5 in Week 6 folder

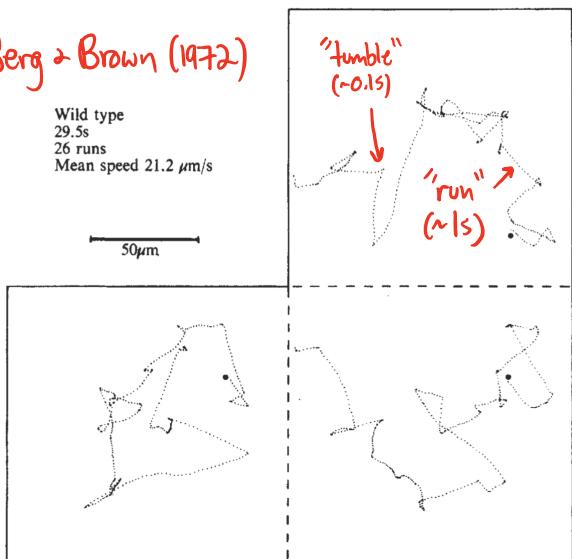
Last time: Introduction to chemotaxis

- ① "Swimming" E. coli cells perform an active random walk

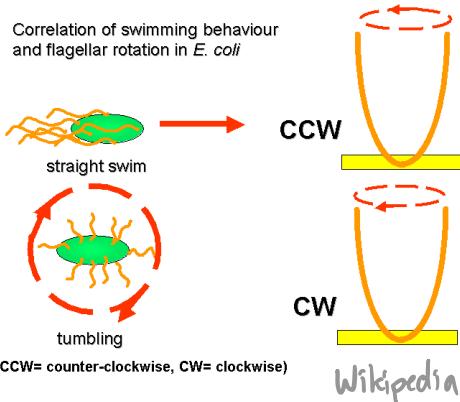
Berg & Brown (1972)

Wild type
29.5s
26 runs
Mean speed 21.2 $\mu\text{m}/\text{s}$

50 μm

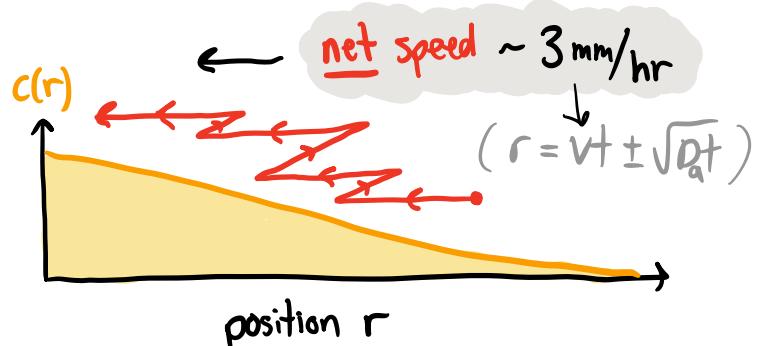
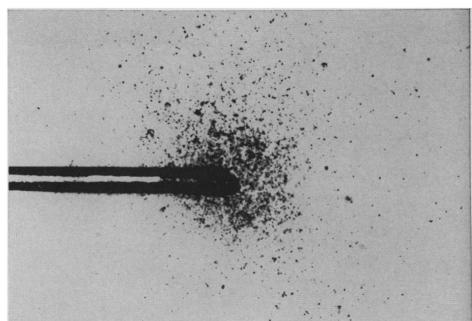


Correlation of swimming behaviour and flagellar rotation in *E. coli*



Wikipedia

- ② Cells swim up concentration gradients by modulating $\text{Pr}[\text{cw}]$

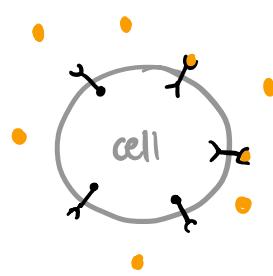


\Rightarrow Today: how do cells sense + respond to environmental concentration gradients?

Simpler question: How do cells measure concentration levels?

⇒ Lecture 3:

Ligand/receptor
binding



$$Pr[X] = \frac{C^n}{K_m^n + C^n}$$

A graph showing the relationship between the probability of activation, $Pr[X]$, and the concentration, C . The curve starts at zero for very low concentrations and approaches a plateau of 1 as the concentration increases, passing through a midpoint at $C = K_m$.

⇒ simplest approach: fraction of active receptors, $f_R^*(c)$
gives readout of concentration level
(e.g. by phosphorylating other proteins)

Problem: chemotaxis works @ extremely low concentrations!

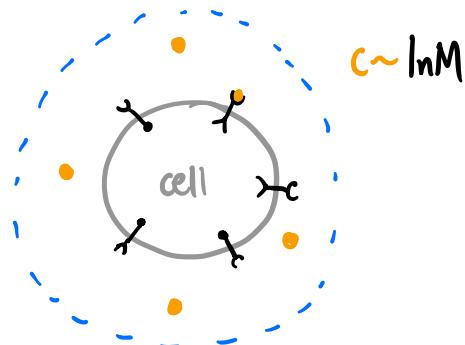
⇒ e.g. $C \sim 3 \text{ nM}$ in microfluidic chambers

(Mao et al PNAS 2003)

How low is this?

$$3 \text{ nM} = \frac{3 \times 10^{-9} \text{ moles}}{\text{L}} \cdot \frac{6 \times 10^{23}}{1 \text{ mole}} \cdot \frac{1 \text{ L}}{1000 \text{ cm}^3} \cdot \left(\frac{1 \text{ cm}}{10^4 \text{ nm}} \right)^3 \approx 2 \text{ nm}^{-3}$$

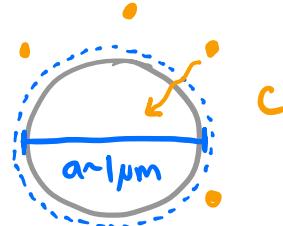
\Rightarrow only a handful of molecules
in neighborhood of cell...



\Rightarrow Additional constraint: "shot noise" in molecular arrivals

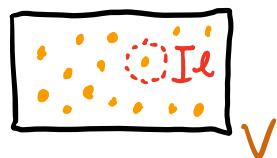
Berg & Purcell ('77): what's the best that E. coli could do?

Idea: maximum # of detected • molecules
eventually limited by diffusion:



① Lecture 5: if wait for time τ , molecules can reach
cell from distance $l \sim \sqrt{D\tau}$ away

② At equilibrium, each of $N = c \cdot V$ molecules
has probability $\sim l^3/V$ of falling in this region



③ Lecture 5: each of these has probability $\sim \frac{a}{\ell}$
of ever reaching cell (vs diffusing away to ∞ ...)

\Rightarrow each of $N = cV$ molecules has probability

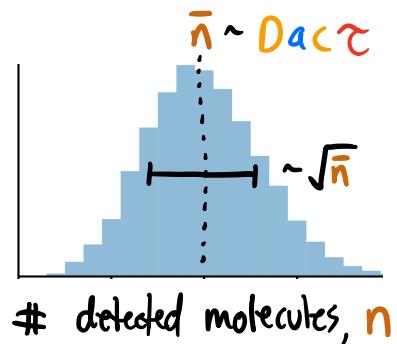
$$P_d \sim \frac{\ell^3}{V} \cdot \frac{a}{\ell} \sim \frac{D_a \tau}{V} \text{ of being detected}$$

④ Actual # of detected molecules (n) = binomial dist'n

(i.e. flip N biased coins w/ prob P_d of getting heads)

Mean: $\langle n \rangle \equiv \bar{n} = N \cdot P_d \sim D_a \tau$

Variance: $\langle (n - \bar{n})^2 \rangle = N \cdot P_d (1 - P_d) \approx \bar{n}$



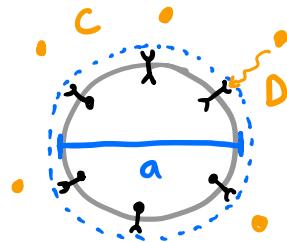
\Downarrow i.e. $n \approx \bar{n} \pm \sqrt{\bar{n}}$ ("shot noise")

\Rightarrow could estimate concentration as $\hat{c} = \frac{n}{D_a \tau}$

$$\Rightarrow \hat{c} = \frac{\bar{n} \pm \sqrt{\bar{n}}}{D_a \tau} = \frac{D_a \tau \pm \sqrt{D_a \tau}}{D_a \tau} = c \pm \sqrt{\frac{c}{D_a \tau}}$$

$$\Rightarrow \text{Relative error: } \frac{\delta c}{c} = \left| \frac{\hat{c} - c}{c} \right| = \frac{1}{c} \sqrt{\frac{c}{D_a \tau}} = \frac{1}{\sqrt{D_a \tau}} \leftarrow \bar{n}$$

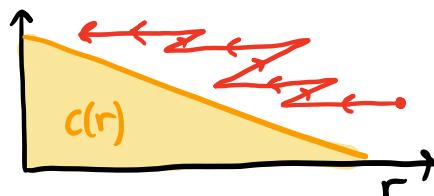
"Berg-Purcell limit" $\frac{\delta c}{c} \gtrsim \frac{1}{\sqrt{D_a \tau}}$
on precision of conc. measurements



\Rightarrow e.g. E. coli sensing $\sim 3\text{nM}$ glucose for $\tau \sim 1\text{s}$:

$$\frac{\delta c}{c} \gtrsim \left[500 \text{ nm}^2/\text{s} \cdot 1\mu\text{m} \cdot 3\text{nM} \cdot 1\text{s} \right]^{-\frac{1}{2}} \approx 3\% \text{ error}$$

Question: How does this limit **chemotaxis**?



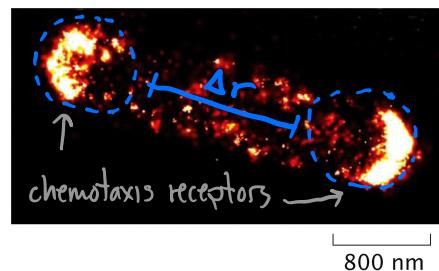
$$\Rightarrow \text{Measured gradient: } \frac{\hat{c}(r + \Delta r) - \hat{c}(r)}{\Delta r} \approx \frac{dc}{dr} \pm \frac{\delta c}{\Delta r}$$

$$\Rightarrow \text{Min. measurable gradient: } \frac{1}{c} \frac{dc}{dr} \gtrsim \frac{1}{\Delta r} \cdot \frac{\delta c}{c}$$

from Berg-Purcell limit

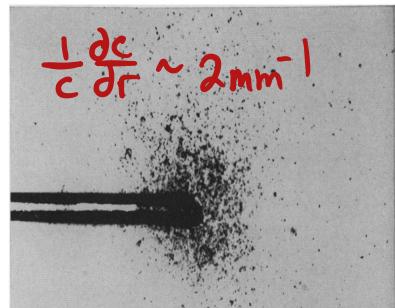
$\downarrow \frac{d \log c}{dr}$

e.g. E. coli measuring gradient across cell ($\Delta r \sim 1\mu\text{m}$)



$$\Rightarrow \frac{1}{c} \frac{dc}{dr} \gtrsim \frac{3\%}{1\mu\text{m}} \approx 30 \text{ mm}^{-1}$$

but experiments much lower...



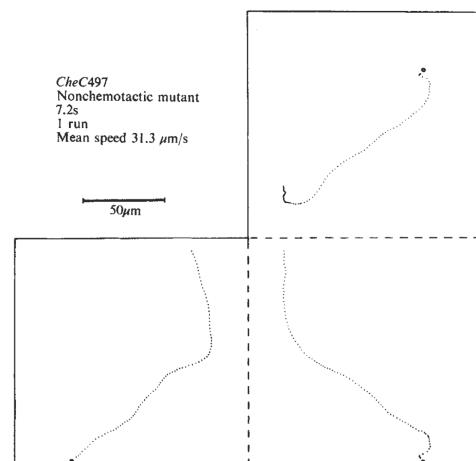
cells would need to count for
 $\tau \sim (15)^2 \times 1\text{s}$
 $\sim 4\text{mins}$

Question: why don't E. coli just measure really carefully @ beginning & swim straight @ target?

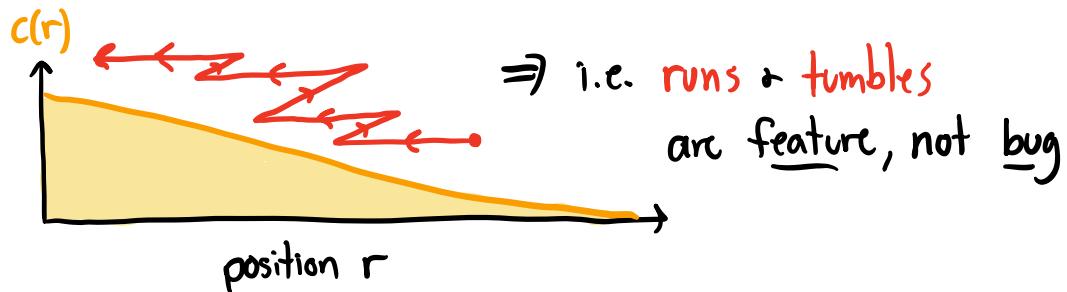
\Rightarrow Rotational Diffusion

$$\text{coli} \quad \langle \Theta(t)^2 \rangle = 2D_\Theta t$$

disorients them after $\tau_\Theta \sim 10\text{s}$



\Rightarrow E. coli must use runs to measure conc. gradients

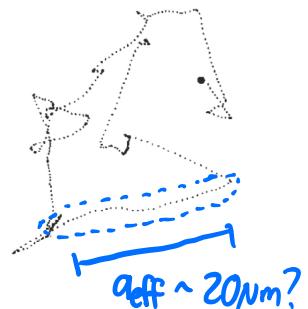


\Rightarrow How does this evade
gradient detection limit?

$$\frac{1}{c} \frac{dc}{dr} \gtrsim \frac{1}{\Delta r} \left(\frac{\delta c}{c} \right)_R$$

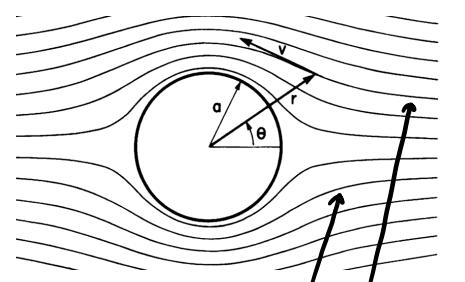
must change
one of these...

Question: does swimming increase
intake of attractant?
(+ decrease $\delta c/c$?)



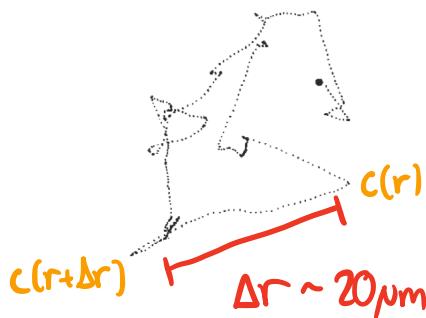
\Rightarrow Berg & Purcell '77: no!

(@ low Reynolds #, E.coli drags)
local neighborhood along w/ it



new fluid pushed out of way...

\Rightarrow Instead: Swimming increases $\Delta r_{\text{eff}} \approx v \cdot \tau$!

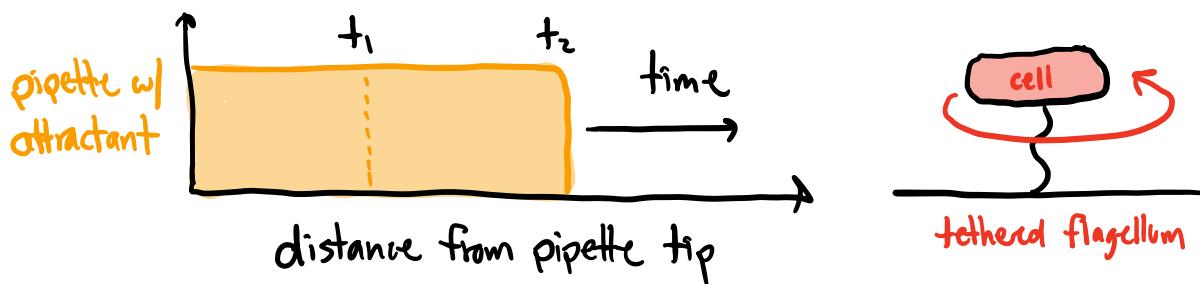


$$\frac{1}{c} \frac{dc}{dr} \gtrsim \frac{3\%}{20 \mu\text{m}} \approx 1-2 \text{ mm}^{-1}$$

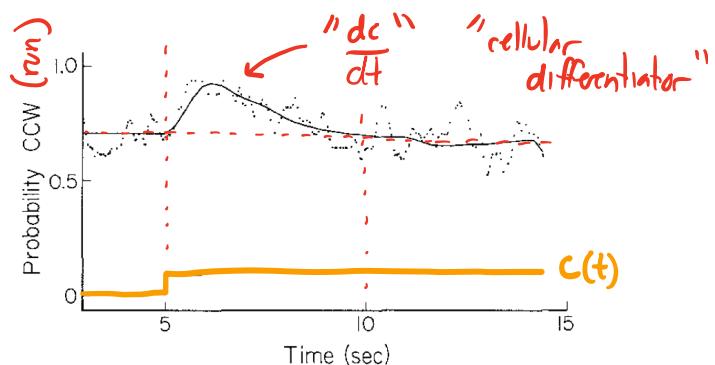
(consistent w/ experimental values ✓)

\Rightarrow cells must be able to measure gradients over time!

Further proof: tethering experiments (Block et al 1983)



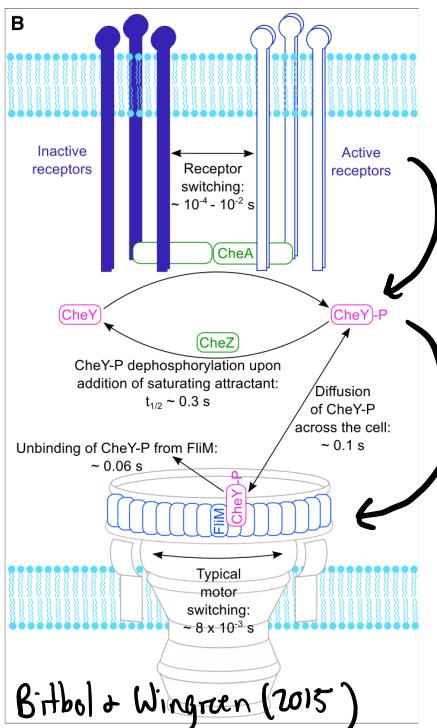
Measured response:



\Rightarrow requires memory ($t \sim 1s$) & adaptation ($t \gg 1s$)
(to calculate over run)

Next: how do real cells actually do this
w/ simple molecular toolbox?

E. coli's Chemotaxis Pathway :



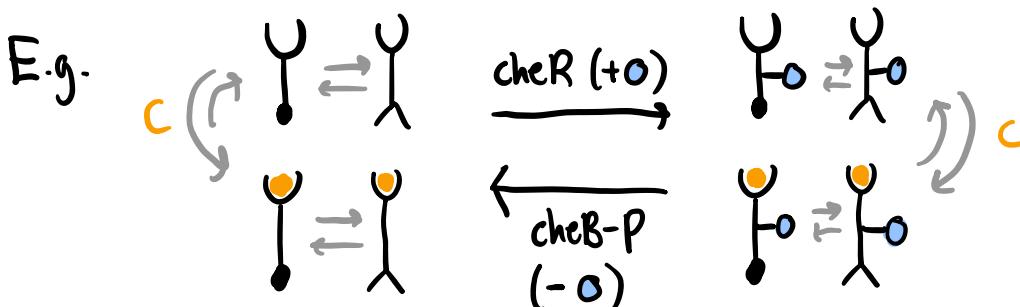
Bitbol & Wingreen (2015)

① active receptor complexes
phosphorylate cheY protein

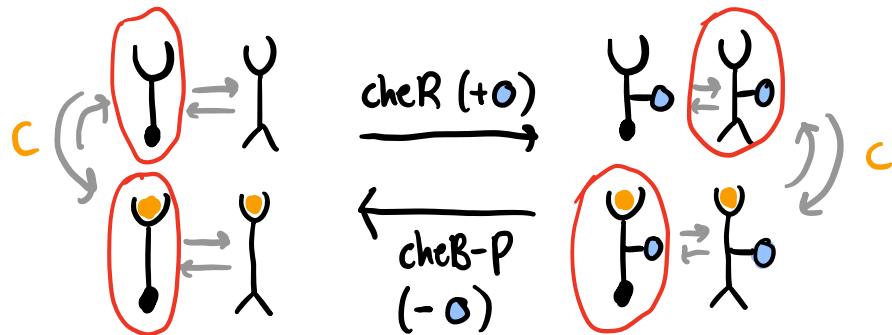
② cheY-P catalyzes switch
to CW motion (tumble)

Upshot: tumbling rate increases
w/ # active receptors

③ Receptor activity controlled by ligand binding
& post-transcriptional modification (methylation)

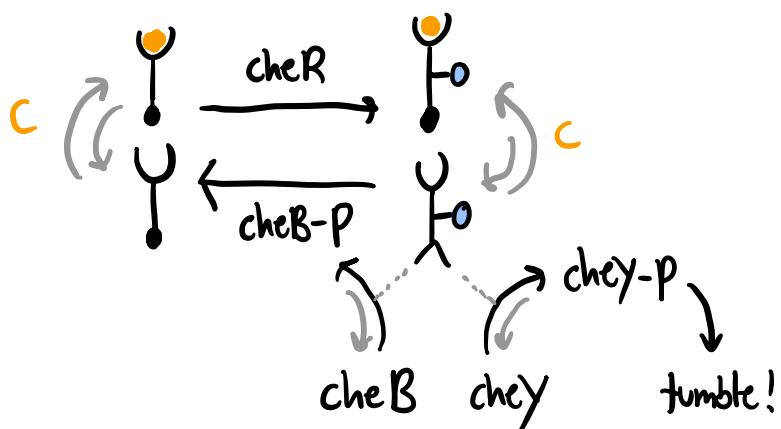


\Rightarrow Free energy landscape favors:

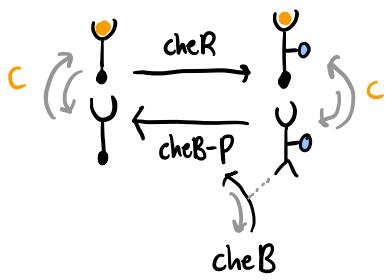


$$\Rightarrow \Pr[\text{active} | \text{X}] \approx 0, \quad \Pr[\text{active} | \text{O}] \equiv f_m^*(c) \quad \begin{array}{c} \uparrow \\ \text{e.g. } K_m^n / (K_m^n + c^n) \end{array}$$

④ Demethylation enzymes (cheR) regulated by receptors!



Minimal
toy model:



phosphorylated cheB proteins.

activated receptors.

free cheB

$$\frac{d[B_P]}{dt} = k_{on}^B [M_A][B] - k_{off}^B [B_P]$$

$\frac{d[M]}{dt} = k_{on}^M [R]_o - k_{off}^M [B_P]$ (saturated methylation enzymes...)

$$[M_A] = [M] \cdot \frac{K_m}{K_m + C}$$
 (chemo-attractant binding = fast)

+ $[B] + [B_P] = \text{const} = [B]_o$ (conservation of cheB)

\Rightarrow @ equilibrium :

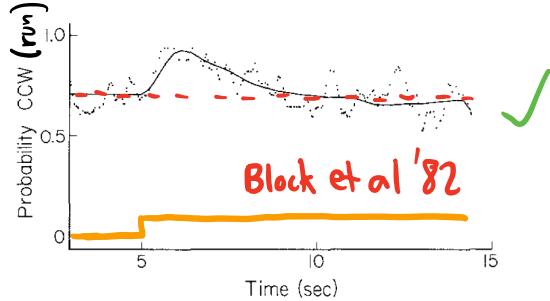
$$\frac{d[M]}{dt} = k_{on}^M [R]_o - k_{off}^M [B_P] = 0 \Rightarrow [B_P]_{eq} = \frac{k_{on}^M}{k_{off}^M} [R]_o$$

$$\frac{d[B_P]}{dt} = k_{on}^B [M_A][B] - k_{off}^B [B_P] = 0 \Rightarrow [M_A]_{eq} = \frac{k_{off}^B}{k_{on}^B} \frac{[B_P]_{eq}}{[B]_{eq}}$$

$$\Rightarrow [M_A]_{eq} = \frac{k_{on}^B k_{on}^M [R]_o}{k_{off}^B (k_{off}^M [B]_o - k_{on}^M [R]_o)}$$

independent of concentration C!

\Rightarrow steady state tumbling
independent of c !



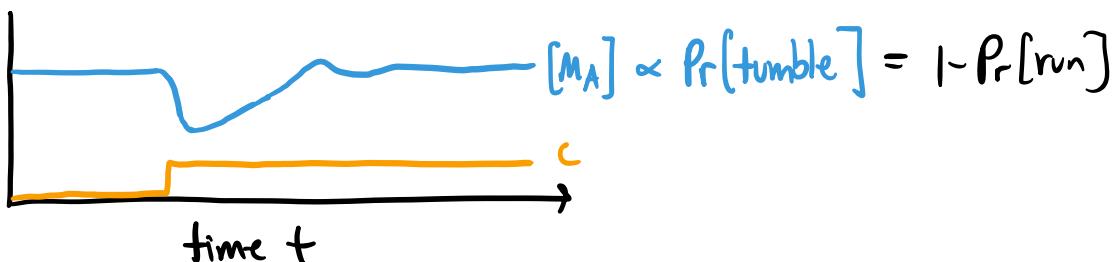
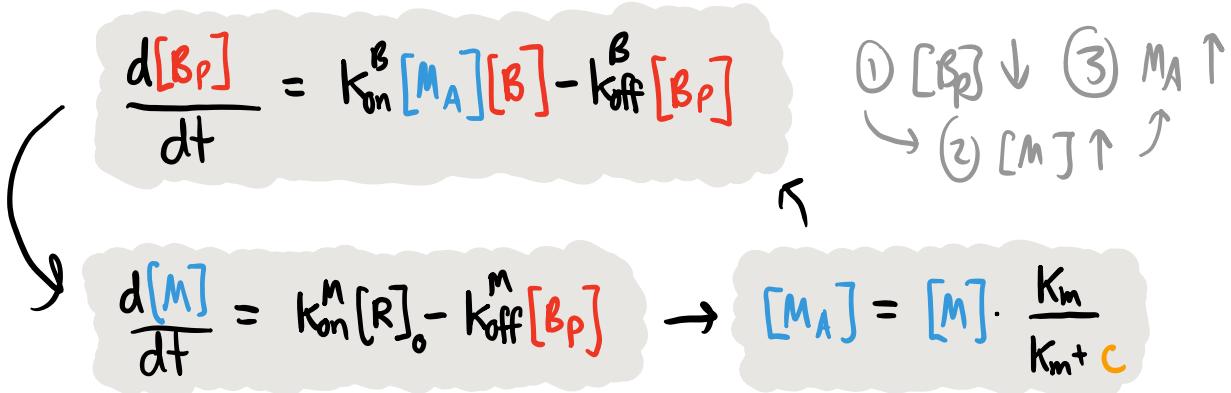
How?

$$[M_A] = [M] \cdot \frac{K_m}{K_m + c}$$

changes in c compensated
by changes in $[M]$

\Rightarrow But takes finite time for $[M]$ to catch up!

E.g. sudden $c \rightarrow c + \delta c \Rightarrow [M_A] < [M_A]_{eq}$



\Rightarrow simple mechanism for measuring temporal gradients!

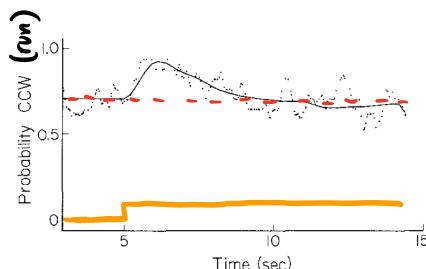
\Rightarrow more realistic version: Tu et al (2008)

Summary:

① Chemotaxis in E. coli strongly constrained by laws of physics!



② "Adaptation" + "memory" can be implemented w/
simple molecular components



\Rightarrow Supplemental Reading (available on Canvas):

① Bitbol & Wingreen (2015) Biophys J

② Tu et al (2008) PNAS